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A COMPARATIVE AND EXPERIMENTAL STUDY OF RACILLI PRODUCING RED PIGMENT.

A DISSERTATION

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Contents.

A. Introductory.

The mechanical difficulties of observing structures so minute as bacteria have led to the accumulation of much differential detail, and to an insistence on all points of unlikeness in physiological characters or cultural reactions. Further, the lack of uniform methods and standards of comparison has tended to produce an overwhelming multiplicity of inadequate species-descriptions, in which any slight variation from existing descriptions, e. g. as regards formation of colonies on gelatin, has been erected into a character of specific importance. But with the publication of Marshall Ward's series of studies on Thames bacteria, of Fuller and Johnston's work on the bacteria of the Ohio River, and of Jordan's paper on some 600 germs found in the Mississippi River, a check has been put to indiscriminate multiplication of bacterial "species". Recently, again, the demonstration of linking intermediate paratyphoid and enteritidis forms in the colon-typhosus group, and of the marked range of individual variation occurring in other organisms, has rendered especially important the questions of variability and of the actual lines between "species", "varieties", and "races".

The chromogenic bacteria afford, from the fact of their pigmentation, a particularly suitable field for observation; and the non-parasitic nature of the special group here considered is another factor favorable for comparative and for variation study. The warping effect of parasitic life upon the physiological and morphological character of organisms is well known, and more characteristic results are to be anticipated from the study of variation in a group of saprophytic organisms than in a group of pathogenic organisms.

The agreement, among a number of bacteria, in a characteristic so marked as is pigment production, might conceivably place a series of red or yellow chromogenic germs in a category by themselves, and raise the question whether this agreement in color production be paralleled by agreement in biochemical and other features, and whether the pigmentation be a fixed, a variable, or a vital character of the organisms. So far, however, attention has not been directed to a series of chromogenic germs, with the exception of the comparative work done by Thumm, by Růžička, and by Jordan on strains of B. pyocyaneus, and of the chemical studies on bacterial pigments in general, referred to below. The best known of the red saprophytes, B. prodigiosus, has been

quite fully discussed since its first descriptions; but no more than two or three of its nearest relatives have at any time been included with it in the study.

The parallel treatment attempted in this paper possesses certain points of value; but comparison of a relatively large number of organisms has its obvious disadvantages as well as its advantages. The number brought under discussion is plainly too great to permit, in limited time and space, of extending the work on each germ into detail, or of carrying out all possible tests, chemical, spectroscopic and physiological. But, on the other hand, the comparison of so many red chromogenic bacteria must throw some light on general features of relationship and variability which are apt to be obscured or lost sight of when attention is concentrated on a single organism or on a small group.

With these considerations in mind, I have attempted comparative study of the following series of forty cultures of red chromogenic bacilli. A few of these, often referred to in bacteriological literature, have been more or less completely studied as individuals, e. g. B. prodigiosus, B. kiliensis, B. lactis erythrogenes; but the majority, though frequently mentioned, have received only the most cursory descriptions. I have therefore prefixed to my comparative notes a brief description of each "species" here discussed. A list of red chromogenic bacilli not obtainable for study will be found at the close of the paper, where are also tabulated the data regarding my series.

As an aid in determining the specificity of some members of the series, I obtained from various laboratories eight cultures of B. prodigiosus; the range of variability thus demonstrated for that species and the other parallels or differences between the allied cultures have suggested my closing note on grouping and differentiation.

Red chromogenic bacilli described in this paper.

B. prodigiosus (Ehrenberg) I—VIII
" " " I, from University of Chicago.
" " " II, from Rush Medical College, Chicago, 1902.
" " " III, from Board of Health, Chicago, 1902.
" " " IV, from University of Minnesota, 1903.
" " " V, from University of Minnesota, 1903.
" " " VI, from Yale University, 1903.
" " " VII, from Ontario, Board of Health, 1903.
" " " VIII, from University of Michigan, 1903.
B. ruber indicus (Koch) I, from Král's Laboratorium, 1900.
" " " " II, from Rush Medical College, Chicago, 1902; obtained by them from Parke, Davis & Company, Detroit, 1899, obtained by the latter from the University of Michigan (?) several years before.
B. ruber plymouthensis (Fischer) I, from Král's Laboratorium, 1899.
" " " " II, (B. No. 18), from air at Cold Spring Harbor, L. I., 1901.
" " " " III, (B. No. 19), from air at Cold Spring Harbor, 1901.
B. kiliensis (Breunig) from Rush Medical College, Chicago, 1902; obtained by them from Parke, Davis & Company, 1899.
B. ruber balticus (Breunig, Kruse) from Král's Laboratorium, 1900.

B. miniaceus (Zimmermann) I, from Králs Laboratorium, 1901
" " " II, from Hoagland Laboratory, Brooklyn, 1899.
" " " III, from Rush Medical College, 1902; obtained by them from Hoagland Laboratory, Brooklyn.
B. rutilus (n. sp.) from the water of the Mississippi River, 1899.
B. amyloruber (n. sp.) from the water of the Mississippi River, 1901.
B. fuchsinus (Boekhout and de Vries) from Král's Laboratorium, 1900.
B. ruber (Miquel) from Král's Laboratorium, 1900.
B. rubricus (n. sp.) from the water of the Mississippi River, 1901.
B. rufus (n. sp.) from the water of the Mississippi River, 1901.
B. ruber (Zimmermann) from Král's Laboratorium, 1900.
B. havaniensis (Sternberg) from University of Chicago.
B. lactis erythrogenes (Hueppe) I, from Král's Laboratorium, 1900.
" " " " II, from the Mississippi River, 1901.
B. rubefaciens (Zimmermann) from Král's Laboratorinm, 1900.
B. lactorubefaciens (Gruber) from Gruber, 1902.
B. rutilescens (n. sp.) from the water of the Mississippi River, 1901.
B. mycoides roseus (Scholl) from Král's Laboratorium, 1900.
B. mycoides corallinus (n. sp.) from the water of the Mississippi River, 1899.
B. latericeus (Adametz) from Král's Laboratorium, 1900, 1903.
B. rubropertinctus (Grassberger) from Králs Laboratorium, 1902; not named by Grassberger.
B. rosaceus metalloides (Tataroff) from Král's Laboratorium, 1900 [1]).
B. mesentericus ruber (Globig) I—IV
" " " " I, from Král's Laboratorium, 1900, 1903.
" " " " II, from the water of the Mississippi River, 1901.
" " " " III, IV, from the water of the Mississippi River, 1901.

B. Historical and Descriptive.

B. prodigiosus is usually taken as a type of red pigment producing bacteria, and is said to be the earliest chromogenic bacterium known. Considering, however, the frequency with which there are found in air different varieties of red pigment germs not identical with, but much like Prodigiosus, the history usually connected with the name of Prodigiosus probably extends to many varieties of this type.

The frequency with which certain of the red chromogenic bacteria appear upon food-stuffs has been a matter of observation for centuries. Lucian, in one of his dialogues (2d century A. D.), makes Pythagoras give, as reason for forbidding his disciples to eat beans, the fact that white cooked beans, if placed in the moonlight, change into blood. Since the forbidding of beans as food is common to various sects of ancient times, e. g. to the Egyptian priests and to the Zoroastrians, from which latter Pythagoras doubtless obtained the notion, the recognition of this pigmentation appears to be of extreme antiquity. In the year 332 B. C. the so-called "blood-miracle" was of service to Alexander the Great in the conquest of Tyre. The bread of his besieging army was discovered to be reddened when broken; but the priests quieted the terrified soldiery by interpreting the omen to mean, that as the "blood" was inside the bread, a bloody fate would fall upon those inside, not outside, the city. For the story see Curtius Rufus, Hist. Alexandri, chap. 2, bk. 4.

1) Previously described by the writer, cf. An unusual bacterial grouping. (Centralbl. f. Bakt. Bd. VIII. 1902. p. 690.)

The phenomenon of the "bleeding host", so often regarded as a miracle in the Middle Ages, was due to a similar cause. The composition of the sacramental bread, rich in starch and poor in acid, was well adapted to the rapid growth of Schizomycetes; but the popular explanation of the phenomenon was that the host had been stabbed by unbelieving Jews. The number of executions and murders due to this belief was so great that Scheurlen, in alluding to it, remarks that "dieser Saprophyt mehr Menschen umgebracht hat als mancher pathogene Bacillus".

Three nineteenth century appearances of this "blood miracle" as epidemic in a town or neighborhood are of interest. In the year 1819, at Legnaro, near Padua, Italy, the whole district was set in commotion by the appearance of red spots upon food. An investigation was undertaken by physicians and professors of the University of Padua, the fungus nature of the growth was discovered, and two separate names given to it. Bizio (23) called the organism Serratia marcescens, the Serratia in compliment to the savant who first propelled a boat by steam on the Arno. His generic description is of interest: — — "Funguli acaules, semisphaerica, capsulis contortis. Serratia marcescens. Vesicula tenuissima, latice primo roseo, dehinc rubro repleta." Bizio reprinted his paper of 1823 in volume I of his Opuscoli chimico-fisico, 1827, and several times later asserted his claim to priority in the investigation of B. prodigiosus. Meanwhile, the same epidemic of Legnaro had been reported by Sette (25), and the name of Zoogalactina immetropa given to the organism. More than twenty years later, in January 1844, there appeared in the Journal de Pharmacie a report by various members of the French Academy of Sciences, who had been commissioned to investigate a reddening of the munition bread which was exciting among the French soldiery much the same agitation as had prevailed among the troops of Alexander. This report, and the treatment of the phenomenon as new, brought out a letter from Bizio (24), emphasizing his work of twenty-four years earlier, and giving the reference to it. However, the next noteworthy epidemic of the sort in Berlin in 1848, investigated by Ehrenberg (26), has not only fixed upon the organism the name Prodigiosus then given to it, but has connected the name of the German scholar with its discovery.

Since Ehrenberg's monograph many investigators have dealt with the biological characteristics and the pigment of B. prodigiosus. A differentiation among the red germs was introduced much later; thus, B. ruber indicus was first described in 1884, B. ruber plymouthensis by Fischer in 1887, B. mesentericus ruber (Globig) in 1888, and B. lactis erythrogenes (Hueppe) in 1889, etc.

B. prodigiosus (Ehrenberg).

This most common of all the pigment forms has been so completely described within the last few years that I can add little

except in confirmation or contradiction of the results already obtained. The following description of my culture B. prodigiosus I, believed to be typical, will be used as a standard for comparison of a series of similar forms.

Morphology.

Ehrenberg described his organism as a monad. Erdmann (28), when it appeared in the cholera epidemic of 1866, called it a bacillus. Schroeter (29) 1872) named it Bacterium prodigiosum, meaning by his use of the term Bacterium that it was non-motile. It was first called a Micrococcus by Cohn (30) in 1872, and was known as such until Schottelius (35) in 1887 described it as a motile rodlet. Wasserzug (37) (1888) thought he could produce the coccus or the bacillus form at will by growth on alkaline or acid agar. In 1896 Scheurlen (43) made a careful description of the morphology of B. prodigiosus; a two day culture in neutral bouillon showed a rod 1,5—2 times as long as broad, with rounded ends. On the long side were 2—4 flagella. On a two-day potato culture, the rods were smaller, ellipsoid, and capsulated. Scheurlen ascribed this variation to the production of unstable alkali (ammonia) by the organism itself. The same thing was true for alkaline media. Finally, Migula (10) (1900) adds, that the bacillus is 0,5 μ broad and from 0,5—1,0 μ long, sometimes occurring in chains of 2—6 bacilli. Motility increases and the rodlike form becomes more marked in slightly acid media. Flagella are peritrichial, but vary in number and length.

Cultural features[1]).

Gelatin. Investigators have not differed as to the growth of B. prodigiosus on gelatin and agar. On a gelatin plate the growth takes place quickly; after twenty-four hours at 20—25° C, the deep colonies appear round, sharply contoured, granular and gray. The surface colonies are thinner and more granular, white, and round or slightly irregular on the edges. On the following day the gelatin is completely liquefied, a red color appears, and later the whole liquid becomes red and cloudy.

In a vigorous gelatin stab culture a deep funnel of liquefaction appears in 24 hours. Pigment appears in 48 hours, and the gelatin is rapidly liquefied to the bottom of the tube and colored red throughout. Usually no pellicle is formed. The rapidity of liquefaction as well as of pigment production varies according to the quality of the medium and the degree of vigor of the cultures.

Agar. The character of the colonies on an agar plate is quite uniform. In 24 hours the colonies appear in the depths, ellipsoid, granular, and reddish. On reaching the surface they spread out round, thinner, and granular, becoming bright red. The color appears first in the center, and under low power the edges are thin, transparent, and finely granular. The diameter reaches 3—7 mm in five days.

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1) Unless otherwise specified, observations were made at room temperature, 18—24° C.

On agar slant the growth appears first as a white smooth moist layer, in 24 hours becoming bright red. In three or four days a green fuchsin-like luster appears on the surface. Old cultures grow darker in color (cf. table in section on pigment).

Potato. This was the culture medium used by the early investigators. The growth and pigment production are here most beautiful, particularly if the potato is fresh and slightly acid. A white line appears in twelve to eighteen hours, which rapidly turns red. The growth is luxuriant and the surface takes on a green metallic luster in a few days, while the red color spreads over the potato by means of the film of moisture. If the potato is dry, growth and pigmentation are limited to the needle track, and the superficial green appears as an abundant, dull, granular layer.

Blood serum. The growth is like that upon agar, but slighter. The medium later undergoes liquefaction.

Bouillon. Ordinary neutral bouillon becomes cloudy in 12 to 18 hours. Later a ring of red color appears at the surface and a flocculent red and white sediment accumulates. The surface sometimes has a slight red pellicle and the whole liquid may be tinged red.

Milk. In milk the red color appears at the surface, the pigment, according to Flügge (3) and Migula (10), adhering to the fat droplets. A soft solid coagulum is formed in 48 hours by the simultaneous formation of "Milchsäure und Labferment" (Gorini (41)). In ten days the coagulum shrinks to half its size, leaving a clear watery serum. Little or no peptonization takes place.

Gas production. The question of the production of gas in sugar solutions by B. prodigiosus has occasioned some discussion. Liborius (34) (1886) described gas development by B. prodigiosus in dextrose gelatine. Schottelius (35) (1887) stated that this organism possessed in a marked degree the power of converting sugar solution into alcohol and CO_2, a statement which has been embodied in Fraenkel's text book (4). Scheurlen, however, disagrees with this conclusion of Schottelius. He found that, in a peptone solution to which 2 % sugar had been added, a gas bubble appeared upon inoculation with B. prodigiosus, did not increase, and, because of its absorption by NaOH, was proved to be CO_2; but he believed this to be the result of the action of succinic acid (which he had shown to be a product of B. prodigiosus in potato culture) upon the sodium carbonate used to neutralize the bouillon. When he tried a sugar peptone bouillon neutralized by sodium-phosphate, or one to which no alkali had been added, or an asparagin-sulphate-phosphate sugar solution, he obtained no gas bubble. The bubble appeared, however, when B. prodigiosus was grown in any medium which had been neutralized by Na_2CO_3, even when the medium contained no sugar. He also obtained gas without the presence of B. prodigiosus merely by the addition of a little succinic acid to the medium. Ritter (47) (1900) confirms Scheurlen, and says

that the gas of Liborius and Schottelius was only a chemical product of the action of succinic acid, which B. prodigiosus forms from sugar upon Na_2CO_3.

My results differ from those of Scheurlen and of Ritter. In standard bouillon, freed from muscle sugar by Theobald Smith's method, neutralized by NaOH instead of by Na_2CO_3, and then having added to it $1^1/_2$ % dextrose, B. prodigiosus I produced at the end of a week a bubble of gas in the fermentation tube. Contrary to Scheurlen's observation, this did not remain stationary but increased daily.

1)	7th	day	4	mm	2)	7th	day	bubble
	8th	„	7	„		8th	„	12 mm
	10th	„	5	„		10th	„	19 „
	12th	„	5	„		11th	„	0 „
	17th	„	5	„				31 mm 34,5 %
	19th	„	0	„				
			26	mm 34,5 %				

All of the gas was absorbed by NaOH, showing that the gas formed was CO_2. Fermentation of the carbohydrate must have taken place, for in this case no sodium carbonate had been added to the medium, from which succinic acid could set CO_2 free. The end reaction of the medium was acid to litmus.

In lactose and sucrose bouillon no gas was produced, nor did any appear in asparagin-sulphate-phosphate solution to which 2 % dextrose was added.

Oxygen relations. Liborius says (loc. cit. p. 172) that such facultative anaerobes as Bac. crassus sputig, Bac. pneumoniae, and Proteus vulgaris, which possess the power to ferment sugar, do this equally well in the presence or in the absence of oxygen. "Der Bac. prodigiosus bietet den einzigen Ausnahmsfall, daß Gärung nur in den sauerstofffreien Kulturen eintritt; aber auch hier ist nicht etwa die Gärung ein Mittel, dessen sich der Pilz bedient, um bei dem Sauerstoffausschluß leben und wachsen zu können, sondern er vermag ebensowohl in luftfreiem Raum zu gedeihen, wenn gar kein gärfähiges Material in Nährsubstrat vorhanden ist." My results on the anaerobic life of B. prodigiosus differ from those of Liborius and agree with those of Ritter. In sugar bouillon B. prodigiosus grows, without pigment, in the absence of oxygen. In sugar free bouillon no growth takes place, although upon admission of air after fifteen or twenty days development will proceed. Liborius' results can be explained by the probable presence of muscle sugar in his so-called sugarfree media. As I have shown above, absence of oxygen is not necessary for gas production.

Temperature relations. B. prodigiosus develops at $37^1/_2{}^0$ C, but without pigment production, which is impeded at a temperature of 35°. Development is prevented by a temperature of 42° C.

Nitrate reduction. B. prodigiosus, when grown in nitrate solution, produces a marked reduction of nitrates into nitrites. Grown in the fermentation tube, in sugarfree bouillon to which 1 % KNO_3 has been added, further reduction is shown by the appearance of gas bubbles at the end of the week.

Odor. The cultures of B. prodigiosus are characterized by a strong odor of trimethylamine, although Scheurlen states that this substance is not formed. It has also been stated that the odor is absent in colorless races (Schottelius). It is absent in non-proteid media.

B. prodigiosus II—VIII.

Seven so-called B. prodigiosus cultures were obtained from various sources (cf. prefatory list) for comparison with B. prodigiosus I. Cultures II and III agreed with the type in all respects and were probably derived from it. B. prodigiosus IV, VI and VII were vigorous cultures showing characteristic liquefaction of gelatin, coagulation of milk, red pigment with green luster on agar and potato etc., but causing gas evolution in sucrose as well as in dextrose bouillon. Agar colonies of B. prodigiosus VII were sometimes spreading and proteus-like. B. prodigiosus V and VIII gave a less brillant pigment of a violet red color without metallic luster. No. V produced a heavy orange red membrane-like pellicle in sugar free bouillon, liquefied gelatin more slowly, beginning with a cup-like depression, coagulated milk only in 72 hours, but showed gas bubbles in dextrose, sucrose, and even in lactose fermentation tubes sooner than any other Prodigiosus culture, i. e. in 48 hours. B. prodigiosus VIII produced a viscid growth in ordinary media, which formed long threads when touched with the needle. It coagulated milk in 48 hours and liquefied gelatin rapidly, but produced no gas in sugar bouillon.

B. ruber indicus (Koch, 50).

Isolated by Koch in India from the stomach of an ape, and, according to Kruse (52), again found by Pasquale in Massua. I was unable to obtain the descriptions of either of these investigators, but that of Fraenkel (51) must correspond closely to the original, since he relates how the organism was sent to the Berlin Laboratory by Koch, between two pieces of filter paper in a letter. It was subjected to all the fumigation which the sanitary police deemed necessary for documents leaving a cholera country, was "durchlocht, gechlort und geschwefelt", but survived. Fraenkel states that it differed from B. prodigiosus in developing pigment at 37°, and in its toxicity for guinea pigs and rabbits.

My two cultures, although evidently distinct for several years (cf. prefatory list), were alike in every respect. They differed from the Prodigiosus type as follows:

Gelatin stab, the liquefied portion colored red only at the surface. Agar, colonies pink in 72 hours, 3 mm in diameter, edges

slightly serrate, later spreading and red with green iridescence. On slant agar and potato, pigment production usually poor, growth luxuriant but dirty white. Unlike any other of the red forms, B. ruber indicus produces pigment more readily on alkaline than on acid or neutral agar (cf. section on special media). Bouillon, pellicle and sediment usually white, but in a peptone and water solution vivid red pigment is formed. Milk, coagulum completely peptonized in 10 days at 37° C. Gas, all CO_2, is formed from sucrose as well as from dextrose bouillon, but none from lactose. Development with pigment takes place at 37° C. Nitrate, reduced to nitrite and further to free nitrogen or ammonia. Odor, not characteristic.

B. plymouthensis (Fischer, 54).

Isolated from water and described by Fischer (1887). The complete description is as follows: — (Ein) "beweglich(er) Bacillus, den ich in der Wasserleitung von Plymouth entnommenem Trinkwasser fand und der sich nicht nur durch seine Gestalt (kleine, dicke Stäbchen mit abgerundeten Enden, kurze Fäden bildend), sowie durch die karmoisinrote Farbe des Pigments von den bisher bekannten, einen roten Farbstoff bildenden Bakterien (Micrococcus prodigiosus, Bacillus ruber indicus Koch, und Bacillus ruber Frank) unterscheidet, sondern auch durch eine stark fadenziehende Beschaffenheit der Kulturen, sowie durch lebhafte Gasproduktion gut charakterisiert ist." Voges (55) (1893) also made some observations on this form, which my results confirm and amplify.

The pigment formed by B. plymouthensis on ordinary media could be distinguished from that of B. prodigiosus only by its less vivid color and tendency to deteriorate into a violet pink. Freshly rejuvenated cultures sometimes showed metallic luster on agar, but, contrary to Voges' observations, not on potato. The "fadenziehende" character, mentioned above, seemed constant in B. plymouthensis I during two years' observation, after which it disappeared. This character also appeared in a culture of B. prodigiosus I, and is present in B. prodigiosus VIII (cf. section on discontinuous variation). The main points of distinction between the Prodigiosus type and B. plymouthensis are (1) slower liquefaction of gelatin beginning with a cupshaped depression (cf. B. prod. V), (2) a vigorous (contrary to Voges) production of gas, 70—78 % of it CO_2, in dextrose, lactose and sucrose bouillon. Gas, 42 % of it CO_2, is also formed in a standard asparagin sugar solution. Cultures of B. plymouthensis I have a strong fecal odor.

B. kiliensis (Breunig), B. ruber balticus (Kruse).

Isolated from water and described by Breunig (56) (1888). A culture from Král under the name B. ruber balticus corresponds to the description of Breunig and to that of Laurent (57), who worked on the variability of the "Bacille rouge de Kiel".

A second culture, B. kiliensis, was atypical only in lack of the characteristic pigment, which was revived on special media to the violet red color without the usual green luster. These cultures differed from B. prodigiosus as follows:

Morphologically larger, rodlets of a young potato culture 2,5—5 μ in length, 0,6—0,8 μ in diameter; rodlets from an old potato culture may reach 8,0 μ in length. Motility appears in 4 hours after inoculation on potato at 35° C, in 24 hours at room temperature. Gelatin is rapidly liquefied, with a thick orange red surface pellicle. Breunig described the growth on potato as at first sealingwax red and later like that of B. prodigiosus. My cultures showed in 24 hours a slight growth of a red violet color, which became luxuriant and darker until, in 10 days, it was heavy, corrugated, and looked like iron rust. Green luster is often seen. Bouillon cloudy, thick orange red pellicle, and red sediment. The production of gas by this form, so far as I can find, has not been previously determined. It occurs readily in dextrose, lactose, and sucrose bouillon, and unlike that of the forms so far described, is only in small part (20—28 %) CO_2. In standard asparagin solution with 1,0 % dextrose, gas to 14,3 % of the tube length was formed, none of it CO_2. Development with pigment occurs at 37° C. The pigment is violet red and lacks the orange red surface growth seen at room temperature. Nitrates are reduced to nitrogen gas. Odor not characteristic.

B. miniaceus (Zimmermann).

Isolated in 1889 from water, by Zimmermann (59), who has suggested that the organism seems identical with Dowdeswell's B. rosaceus metalloides. Zimmermann described it, however, and gave it the above name. Migula, commenting on this form, said: "Zimmermanns Vermutung, daß diese Art identisch mit B. rosaceus metalloides ist, dürfte nicht richtig sein. Er schließt sich eher an den Kieler Bacillus an."

Hoagland Laboratory, Brooklyn, furnished a culture, isolated from water, which seemed identical with the B. miniaceus from Král, except for a tendency to lose power of pigment production. A second culture from Hoagland Laboratory, evidently identical with the first, came into my hands as "B. rubrus", was colorless, and could not be made to regain the chromogenic power. This culture produced the typical amount of gas, 40—45 %.

My cultures of B. miniaceus are more like B. plymouthensis than like B. kiliensis. Contrary to Zimmermann, I find the bacillus motile in young cultures. Liquefaction of gelatin, slower than B. plymouthensis, but not so slow as stated by Zimmermann, i. e. begins within 5 days, and is complete in three or four weeks. The liquid is red. Potato and agar, like B. plymouthensis, metallic luster rarely seen and only on glucose agar. Gas production like B. plymouthensis. Nitrates reduced to nitrites only. No fecal odor.

B. rutilus (n. sp.).

This organism was isolated in November, 1899, from the Illinois River. One ccm of water was incubated for 24 hours in 0,55 % carbol broth, then plated in litmus lactose agar, where such a production of pigment occurred that the plate was brilliantly colored. The red pigment form grew luxuriantly upon isolation, and, since it differed from B. prodigiosus and from the others of the series, is here described as a new species. Its vigor of growth and pigment production have been slightly lessened by two years of cultivation upon laboratory media. Its points of difference are as follows:

A short, actively motile bacillus, slightly larger on all media than B. prodigiosus, but smaller than B. ruber balticus. Gelatin, growth and liquefaction rapid, little pellicle, the whole liquid vivid red. When first isolated, agar plate colonies always showed pseudopodia-like ramifications; later, branching more rare, or only on plates from old cultures. Growth and pigment best on acid agar. No metallic luster ever seen on agar or potato. In acid bouillon, the whole liquid colored red, no pellicle. The presence of dextrose and lactose increases the pigment, that of sucrose does not. Milk, acid and coagulated in 24 hours, later peptonized at 35° C. Gas production, in dextrose and sucrose bouillon, when first isolated, 90 % of tube length, 65 % of this CO_2. Later, 50 % of tube length, all CO_2. Nitrates reduced only to nitrites. Odor, like B. prodigiosus.

B. amyloruber (n. sp.).

An organism which differed from B. prodigiosus, B. ruber balticus etc., in pigment and in some other characters, was isolated from Mississippi River water in 1901; because of its ability to grow actively upon starchpeptone media it has been given the above name. After passing through a summer upon neutral agar the character of its growth was somewhat changed, tending to a thin crusty or granular growth instead of one soft and luxuriant. The pigmentation on ordinary media has undergone no deterioration, remaining deep violet red. The chief points of deviation from the forms already described are as follows:

Upon ordinary media the pigment is deep violet red, taking orange color only on alkaline agar. Sugar free bouillon, little pigment; sugar bouillon, colored deep violet red. Milk, peculiar; at first, no change; later, a violet red coloration; after 15 days, a fine red and white sediment of pigment granules and precipitated casein, but no coagulation and no peptonization. No gas is evolved in any sugar bouillon. Nitrates, reduced to nitrites.

B. fuchsinus (Boekhout and de Vries).

This name has been given to two different organisms; 1) to a "new chromogenic bacillus" described by Boekhout and de Vries in 1898 (60), and 2) to the red bacillus described but unnamed by Lustig in 1893 (8), named by Migula in 1900 (10). Lustig's organism, which he described very completely, differs morphologically from B. prodigiosus in that it is a rodlet two

or three times as long as broad, and contains pigment granules in the cell body. Its cultural features are like those of B. prodigiosus, with the exception of pigment production in the absence of oxygen. This organism is called by Kruse (3) B. ruber aquatilis.

Král informed me that the B. fuchsinus sent out by him was obtained from Boekhout and de Vries. According to the description of these authors, their bacillus differs from B. prodigiosus in the following points: It is slightly longer, i. e., 1—1,5 μ long. They describe it as non-motile, but later state that in "Malzagar" it is longer and shows motility. It does not show the metallic luster on ordinary agar, but does on potato and on sodium tartrate agar. (B. prodigiosus often does not show it on agar.) It peptonizes the casein in milk, and does not produce gas in any sugar bouillon.

The culture which came to me from Král showed no pigment upon arrival, nor was I able to induce any pigment production by various methods of rejuvenation, such as alternating series of bouillon and gelatin cultures, growth on potato or on sodium tartrate, agar etc., although the growth was rapid and luxuriant on all media. After cultivating the organism for over a year, I had begun to suspect that this form either was not B. fuchsinus, or had lost its power of pigment production permanently, when I discovered on a culture made on old dry agar a trace of red at the top of the agar slant, where the agar was dryest. By careful transference through a series of old dry agar tubes, I was able to increase the pigment until it showed on the upper third and all around the edge of the white agar growth, and also at the top of growth on dry potato. This pigment was promptly lost, however, when inoculation was made upon fresh media, and never showed in gelatin or bouillon. My culture differed from the description of Boekhout and de Vries only in absence of pigment, and in non-peptonization of casein [1]). Nitrates are reduced to nitrites and to free nitrogen gas. Gas is not formed from sugar bouillon.

B. ruber (Miquel).

This organism was sent to me from Král's Laboratorium; the only description of it is contained in the following very kind answer by Miquel to an inquiry concerning the organism: — „Je n'ai jamais publié de travail sur le Bacillus ruber que vous mentionnez . . . . Le bacille, qui possède les principaux caractères du Bacille rouge de Kiel, mais qui en diffère par sa faculté négative de liquifier la gélatine, fut donné comme curiosité à l'institut Pasteur d'où il a émigré un peu partout jusqu'au Laboratoire de Dr. Král sans être précédé d'un signalement quelqu'un. Si le hazard me le faisait de nouveau rencontrer dans les eaux, peut-être en ferai-je l'histoire en raison de la beauté de son pig-

1) As this paper is appearing in the Centralbl. f. Bakt. I receive through the kindness of Dr. Boekhout a culture of B. fuchsinus showing luxuriant red pigment.

ment moidoré; mais actuellement je n'en saurais dessiner une monographie précise; mes recherches sur ce micro-organisme datant de plus de 10 ans." (1901).

I have completed Miquel's description as follows:—

Morphology, a rodlet 2—3 μ in length, often in chains of several bacilli, non-motile, sporeless. Gelatin colonies, 48 hours, small, irregular, finely granular, with well defined edges; 72 hours, show a peculiar corrugated, overlapping growth; the next day pigment appears at the centre, and later they become deep violet red. In gelatin stab, a red surface colony and a white needle track growth; no liquefaction. 24 hours, surface colonies on agar show a peculiar lined and cracked appearance under low power; in 48 hours they are tinged red, and later look like B. prodigiosus colonies. Agar streak shows green luster and is like B. prodigiosus or B. ruber kiliensis. Potato violet, red line in 24 hours, later darker, often green luster. Does not spread on potato. Blood serum not liquefied. Milk, no change except pigmentation. Gas, 45 % of tube length in dextrose bouillon only, 79 % of gas CO_2. Oxygen and temperature relations, like B. prodigious. Nitrates reduced to nitrites. No characteristic odor.

B. rubricus (n. sp.).

In the autumn of 1901 three cultures were isolated in this laboratory (University of Chicago) from the Mississippi and the Missouri Rivers, which produced red pigment but showed marked differences from B. prodigiosus and the cultures previously described. These cultures do not grow rapidly, and the pigment appears very slowly, the color beginning as pink or salmon pink, and not attaining the characteristic brilliant red for some weeks. Morphology, a small, slender, non-motile bacillus. Colonies, gelatin and agar, slow growing, small, round, non-characteristic, under low power finely granular; color, yellow orange, deepening to red. When first isolated, began to liquefy gelatin very slowly in ten days. After a year's cultivation, the power of liquefying ordinary gelatin had entirely disappeared. Agar slant, when dry and growth limited, may develop bright red pigment in a few days; when moist and spreading, may remain white or light pink for weeks, gradually deepening in color. Potato slight or no growth. Bouillon, cloudy, with pink pellicle. When first isolated, milk unaffected; later, litmus milk cultures showed marked alkalinity. No gas, nitrate not reduced, aërobic. May grow at 37° C, but better at room temperature.

B. rufus (n. sp.).

Two cultures, much like B. rubricus, were isolated at about the same time and place. In pigment and in manner of development this organism was hardly distinguishable from B. rubricus, but differed in showing luxuriant growth on potato, and in not losing its power of liquefying gelatin slowly but completely. Milk, unchanged or slightly alkaline in 15 days.

B. ruber (Zimmermann).

This form was isolated from the Chemnitz water supply and described by Zimmermann in 1890 (61). Migula gives it the name B. pseudoruber, since, he says, the name ruber had already been used by Frank, whose organism, he thinks, is not identical with that of Zimmermann. Zimmermann himself had mentioned Frank's form, but remarked, that identity with his was impossible to determine because of the incomplete description of the former; he thinks that his organism is more likely to be identical with Eisenberg's red bacillus.

My culture of B. ruber Zimmermann, from Král, differs both from the original description and from that of Eisenberg's bacillus (cf. Table X). It is non-liquefying, non-gas producing, and non-luxuriant on potato. It differs from B. rubricus and B. rufus in being an actively motile bacillus. Bouillon shows a peculiar growth; no development is visible for three or four days, then a thin pink pellicle forms on the surface which sends floating cobwebby streamers down into the clear liquid. This may still be seen after 26 days. Some pin-point pink colonies attach themselves to the wall of the tube. Potato shows a slow clear red growth, limited to the needle track. Litmus milk becomes blue through alkali production. No development occurs without oxygen, nor at 37° C. Blood serum not liquefied, nitrate not reduced.

B. havaniensis (Sternberg).

Sternberg has unfortunately given this name to two different organisms; one, the color producing, he termed B. havaniensis (62), the other, colorless, B. havaniensis liquefaciens. Kruse and Migula describe the latter under the name B. havaniensis, which properly belongs to the red form. Sternberg's description of the red form is as follows: — A short oval bacillus, usually in pairs, about 0,4—0,5 μ in diameter. The cells are nearly spherical. It is an aërobic, non-liquefying, chromogenic bacillus, which grows slowly at room temperature. On gelatin plate the colonies are spherical, translucent, of a blood red color; on gelatin stab there is an opaque carmine layer, with a scanty colorless growth in the depths. On agar the growth is slow but continuous, of a glistening red color, with wavy outlines. The organism frequently fails to grow on acid potato, but sometimes develops on old dry potato. Pigment is found only in the presence of free oxygen. — Migula, in describing this organism, does not include it among the red forms; he says that its growth on agar is brown, and that it shows no growth on potato.

The culture here described is of unknown origin, having been in the laboratory (University of Chicago), some years under the name B. havaniensis. It agrees exactly with Sternberg's description as far as that goes. Growth in bouillon is similar to that described for B. ruber Zimmermann. Litmus milk is un-

changed except for orange pigment at the surface. No gas is produced; growth but no pigment occurs at 37°. It differs very little from B. rubricus, i. e., only in bouillon and in milk.

B. lactis erythrogenes (Hueppe).

It was long ago observed in dairies that so-called bacterial "red milk" occured in one of two ways; either red flakes appeared on its surface, or the whole mass of liquid was colored red. The former phenomenon is ascribed to B. prodigiosus; the second is usually due to an organism discovered in 1886 by Hueppe, and described in 1889 by Grotenfeldt, (63) as B. lactis erythrogenes. This organism, according to Grotenfeldt, is a small rodlet, 1—1,4 μ long, non-motile and sporeless. The gelatin colonies are small, round, and at first grey-white, later yellow, as the gelatin becomes liquefied. Around them the gelatin has a rose tint. A gelatin stab culture liquefies slowly from the top. After 10—12 days the liquefied portion is red, with a yellow sediment, while the solid portion below is a weak rose color. The growth on potato and agar is yellow, and after 6—8 days at 37° C, the pigment becomes a deep golden color. Bouillon is cloudy and yellow. Milk undergoes a slow precipitation of casein, leaving a clear serum which becomes blood red; the reaction is alkaline. The organism is not pathogenic; the cultures are characterized by a sweet odor.

My culture of B. lactis erythrogenes, from Král, agreed closely with this description. Another culture, isolated in November 1901 from Mississippi River water, corresponded to the Král culture in all characters except that the color of the yellow pigment was a shade lighter, with a tendency to appear much later in the growth. The following details may be added to the description of these cultures: Growth on agar is moist, luxuriant and yellow, while the medium becomes rose colored as in gelatin, and even wine red. The yellow growth on potato shows often a slight pink tinge, the potato becoming dark and discolored. Blood serum not liquefied; no gas in sugar bouillon; no development without free oxygen; development with pigment at 37° C; nitrates reduced to nitrites. The sweet odor of the cultures is very noticeable.

B. rubefaciens (Zimmermann).

Isolated from the Chemnitz water supply and described by Zimmermann in 1890 (67). Characterized by a rose red color which diffuses throughout solid media such as gelatin or agar. Morphologically, a thin rodlet, 1—1.6 μ long, often in chains of two or three. Actively motile, no spores. Gelatin colonies appear as minute white dots in 48 hours; under low power, pearly, thinner at the edge, which is well defined, finely granular. In gelatin stab, small spherical white colonies occur along the needle track, giving a characteristic appearance. The surface colony is yellowish, and in old cultures a red tinge may be seen in the medium. No

liquefaction. Agar colonies, not characteristic in ten days, small, white, with fragmented edges. An agar slant culture shows a white, smooth, shining, somewhat luxuriant growth. In old cultures the medium takes on a distinct wine-red color, clear and transparent. Zimmermann does not specify this as true for agar, only for gelatin and potato. A heavy cream white layer forms on potato, which later becomes yellow brown. I observed no rose color here, but a brown discoloration of the potato. Bouillon becomes cloudy with a white surface pellicle; after 6—8 weeks it may show a slight reddish tinge. Litmus milk is decolorized and acid, but coagulates at the end of ten days only on boiling. No gas is formed, no growth at 37° C, nitrate reduced to nitrite, facultative anaerobe.

B. lactorubefaciens (Gruber) (68).

Received from Gruber in November, 1902. Upon examination it agreed closely with the original description. I found however, that the deep growth in gelatin was sometimes arborescent, and that the medium was tinged red. On agar and potato, a white spreading growth. Bouillon is turbid, with pellicle and sediment. The colonies are coli-like. Milk is made rose colored and very slimy; litmus milk shows slight acidity, but does not coagulate at room temperature. The organism does not develop at 37° C, which fact, together with its behavior in milk, its lack of yellow pigment, and its motility, distinguish it from B. lactis erythrogenes.

B. rutilescens (n. sp.).

Isolated from Mississippi River water in 1901. Its characters connect it both with the Prodigiosus group and with the Lactis erythrogenes group. Morphologically, an actively motile bacillus, like B. rutilus and B. rubefaciens, unlike B. lactis erythrogenes. No spores. Gelatin plates show white, non characteristic colonies which soon liquefy the gelatin. A stab culture is liquefied rapidly, with a white cumulus sediment, and a white pellicle. Later the liquid portion becomes a beautiful clear rose, and the floating pieces of sediment take on a pink tinge. Agar colonies are often spreading, like those of B. rutilus or B. ruber indicus, without pigment. Agar slant shows a luxuriant, smooth, moist, white growth, much like the pigmentless culture of B. fuchsinus. Potato, luxuriant, white, and spreading. Bouillon, marked turbidity, with white sediment and thin pellicle. Litmus milk, at room temperature slightly acid, coagulating in three days. At 37° C, coagulation in 48 hours, followed by partial peptonization. Gas production, negative; facultative anaerobe, nitrates reduced to nitrites. Unlike B. rubefaciens, grows rapidly at 37° C.

B. mycoides roseus (Scholl).

What seems to be the original description of this organism is

a footnote to Grotenfeldt's article on B. lactis erythrogenes (69) (1889), mentioning a bacillus isolated from earth by Scholl, and studied in the laboratory of Hueppe at Wiesbaden. The bacillus formed red felt-like colonies on gelatin, causing liquefaction. The gelatin had a red pellicle, but was not colored throughout. Agar cultures were red in the dark. It grew quickly at room temperature, and was morphologically like the anthrax bacillus.

Eisenberg (70), Kruse (3), and Migula (71) repeat this description, the latter two authors adding only data as to solution and spectrum analysis of the pigment as determined by Schneider (19). Macé (9 p. 256) mentions the organism. These are the only descriptions that I have found.

My cultures, I and II, were identical in all reactions tested. Morphologically B. mycoides roseus is usually smaller than the anthrax bacillus, but like it variable in size, rodlets from 2—10 μ long occurring in the same culture. In very old agar cultures, short, thick, often coccuslike, non-motile, no spores. Gelatin colonies are characteristic. They appear in three days as minute white dots, under low power showing an opaque center and edges broken or fringed as though crystallized; later the edges lose their fringing and become smooth. In ten days the colonies are 3—5 mm in diameter, somewhat creased and corrugated; they form slight depressions in the gelatin, but do not actually liquefy it. Gelatin stab culture develops slowly a slight needle growth and a dry, thick, corrugated pink colony on the surface. The needle growth is often finely arborescent, like that of the colorless B. mycoides figured by Lehmann and Neumann (7). Agar colonies appear in 48 hours; under low power they show the characteristic fringed edges seen in gelatin colonies. At five days they are pink and small to the eye, less than 1 mm in diameter, edges smooth and granular. Agar slant cultures show little development before 48 hours or three days. Then a salmon pink growth is seen, which becomes dull, dry, and wrinkled, deepening in color as it gets older, finally becoming vermilion. Potato, growth very slow, no development for three or four days; at the end of ten days small orange dots, or if the potato is kept wet, an elevated, warty, moist, salmon pink growth. In 25 days a thick raised layer is seen, which is more orange in color than on agar. Bouillon, slow development, no turbidity. On the third day small round pink colonies appear at the surface; at the end of ten days the surface is covered with a thick agglomeration of salmon pink colonies, some of which sink to the bottom as sediment. Milk, at the end of ten days, shows only the characteristic salmon pink colonies floating on the surface; after 20 days, decidedly alkaline. No gas, no growth in the closed arm; growth and pigment nearly as good at 37° C as at room temperature; nitrates reduced to nitrites.

B. mycoides corallinus (n. sp.).

This organism was isolated from Mississippi River water in

1900, and has since been kept in stock in this laboratory (University of Chicago). Two years of artificial cultivation have somewhat changed its character, but it continues to show several seemingly constant differences from B. mycoides roseus, its resemblance to which has led to the adoption of the name.

When first isolated, young cultures showed a large anthrax-like bacillus, non-motile, sporeless; later the organism became somewhat diminished in size. Gelatin colonies are characteristic, but differ from B. mycoides roseus. They appear in three days as minute points, which under low power have a peculiar woolly, wispy look; as they develop, they become pink, smooth, and raised. Gelatin stab, slow development; a ten day growth is very characteristic, a fine feathery development along the needle track, and a raised, smooth, shining pink surface colony. The minute agar colonies of 48 hours show microscopically the characteristic wispy fibrillar branching; high power shows that the branching is made up of transparent beady ramifications extending in all directions. The colonies are larger than those of B. mycoides roseus of the same age. Agar slant culture is unlike B. mycoides roseus in being smooth, moist, and salmon pink in color. Growth on potato differs in the same way, the color deepening in 20 days to red. In bouillon cloudiness appears in 24 hours; later the turbidity increases, and a pellicle of small separate pink colonies covers the surface, falls as sediment on shaking, and is re-formed. Litmus milk shows strong alkali production and characteristic pigment. Otherwise like B. mycoides roseus.

B. latericeus [?] (Adametz, 72).

I could not obtain the original description of this organism. Migula (10) describes it as a non-motile rodlet, 3—5 times as long as thick, slow growing and non-liquefying. Chester (1) adds that the growth on agar is limited, moist, glistening, and reddish-brown to yellowish-brown; bouillon is clear and alkaline, with stringy sediment; potato, thin, moist, and reddish; litmus milk, decolorized and coagulated; indol, negative; no growth at 36° C. Lehmann and Neumann (7), who give a fuller description, with plates, say that milk is not coagulated, the growth is from vermilion to reddish brown; no gas nor acid is formed from sugar, but traces of indol occur. Their culture was isolated from air and identified from Eisenberg's description (2).

My culture of B. latericeus, obtained from Král, does not agree with any of the above. Dyar (73) also notes the aberrancy of the culture which he received from the same source. Dyar's culture, to which he gives the new name, B. fuscus pallidior, agrees with that here described.

A short, non-motile bacillus, 1—1,3 μ long, and 0,5—0,7 μ broad, occurring single and in chains. Gelatin colonies after four days small, granular, with slightly irregular and ragged edges. Stab cultures show a slight needle growth, and on the surface a

slow growing dry white colony, which after weeks lines a cup-like cavity with a thin dry spreading cream pink growth. No other evidence of liquefaction occurs than the cup shaped depression. Agar colonies are dry and shining to the eye; under low power the edge is ragged, showing the individual rodlets pushing out into the surrounding medium, Agar slant and potato show at first a dry shining whitish layer, which later becomes "crusty", wrinkled, and dull pink. Bouillon remains nearly clear, development taking place in a thick surface pellicle of paraffin-like consistency, from which flakes fall to the bottom of the tube on shaking. Litmus milk, not coagulated, but shows intense alkaline reaction and peptonization after a few weeks at 35° C. Aerobic, non-gas producing, nitrates reduced to nitrites, slight growth at 37° C.

B. rubropertinctus.

In his experiments with acid-resisting organisms, Grassberger (74) made, in 1899, from butter and from guinea pigs inoculated with butter, six isolations of the bacillus described below. My culture, obtained from Král in 1901, agress in biological characters with Grassberger's original notes. As he gave it no name, I have called it on account of its staining reaction, B. rubropertinctus.

Grassberger describes a 24 hours agar culture as showing rods 1,5—3,0 μ long, having no trace of acid resistance when stained. Older cultures occasionally show longer bacilli, which retain the stain slightly when treated with 3 % acid alcohol, but never evince the true tubercle bacillus reaction. I found that a ten day potato culture, when stained on a cover glass together with B. coli by hot carbol fuchsin, washed several times with 10 % H_2SO_4, and counter stained with methylene blue, resisted the acid perfectly, remaining bright red in contrast to the blue B. coli. The bacilli could not be mistaken for B. tuberculosis, being much shorter and thicker.

Gelatin plate shows characteristic colonies, the deeper ones ovoid in shape and granular, the superficial colonies irregular, with crenate edges, folded surface, and red color. Stab culture, slight needle growth and orange red surface colony as above, no liquefaction. Agar colonies, very slow, being just visible to the naked eye in five days; uniformly granular, and irregularly triangular. On agar slant, growth lustreless, dry, and spreading, later wrinkled and vermilion red. Grassberger records one culture that was moist. Potato development, slow but constant; at the end of ten days orange red, rough, granular, and moist. Bouillon, clear save for slight cloudiness at the top; a salmon pink pellicle is formed, which is constantly renewed as it sediments on shaking. Litmus milk shows no change except for the development of a pink pellicle; B. rubropertinctus is not gas-producing, grows at 37°, is aerobic. Grassberger notes slight indol production and cauliflower-like odor. My culture did not show these characters.

B. mesentericus ruber (Globig).

Isolated by Globig, 1888 (75), from potato. Described as a slender, motile, bacillus, with oval, very resistant, spores. Gelatin colonies, round and yellow in the depths; on the surface showing a fringe or halo of fine network, which breaks down in 3—4 days and liquefaction begins. Gelatin stab, funnel shaped liquefaction. Agar colonies are non-characteristic. Agar streak, dirty white and slightly wrinkled growth. On potato a dry growth of a beautiful pink color. Globig described bouillon as clear, with a thick pellicle; my cultures showed cloudiness and pellicle. Litmus milk, curdled and acid. No gas is produced, nitrates are reduced, growth with rose color at 37° C.

Two cultures from Král, 1900, 1903, differed from the above description in not showing spores. Three cultures isolated from the Mississippi River water at different times were used as the basis of this description.

C. Comparative and Experimental Study.

I. Color determination of bacterial pigment[1]).

The increasing tendency towards the adoption of definite terms of positive, negative, or quantitative value in bacterial descriptions has not been generally extended to the determination of color produced by bacteria. The pigmentation of an organism still furnishes an opportunity for a more or less lax and undiscriminating nomenclature. Thus, among the red series one whole classification has been made on the basis of such divisions as these: "Pigment carmine", "pigment flesh-colored," "brick-red", "reddish", "rose-colored", "salmon-pink", "yellowish-reddish", "brownish-red", "pinkish", "reddish-pinkish", "blood-red", "red-brick-red". These distinctions may call up well defined color pictures to individual workers, but it is evident that there are difficulties in the way of fitting a new chromogenic organism to such an imaginative scheme.

In default of a more exact method, statistical biologists have for some time employed a color top[2]), upon which discs of the primary colors may be arranged, showing sectors of definite proportions; when the top is rotated rapidly the colors blend and give the intermediate tints. Any color may thus be matched, and the percentage of primary colors which go to make it up very closely determined. This method is easily applicable to bacterial colors, and has been adopted in this study. Other color-terms appearing

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1) The chemical nature of bacterial pigment, its solubility, and its spectrum analysis has been discussed at length by Schröter (29), Cohn (30), Griffiths (39), Scheurlen (43), Schneider (19), Rosenberg (45), Kraft (49), and others.

2) The Milton Bradley color top has standard colors of the following wavelengths: Red, 656—661; orange, 606—611; yellow, 577—582; green, 514—519; blue, 467—472; violet, 419—424. For method cf. Davenport, C. B., Statistical Methods. New York, 1899, p. 9.

here are either quoted or applied to the culture media in distinction from the bacterial pigments.

One difficulty, however, in determining bacterial pigment, is the possible range of variation in any one organism under influence of preliminary cultivation, reaction of media, and age of culture; so that one color-determination may be insufficient for general description. Neither can the mean of several determinations be specified as the typical color of a given organism. But, allowing for occasional degenerate or pigmentless cultures, if an organism has been put through the regular course of rejuvenation and then grown on slant agar of definite composition and reaction, the pigment of young and of ten day old cultures can be fairly well characterized. The appended tables will give an idea of the results of this method; and the terms used in this paper to designate the different reds will have definite meaning as follows:

	Red	Orange	White	Blue
Vermilion	30	70		
Orange red	45	55		
Salmon pink	50	25	25	
Pink	50	25	22	3
Violet red	80	15		5
Red	90	10		
Dark red	100			

(see Table I p. 22.)

Of the cultures producing "insoluble" pigment on agar, which are studied here, a division may be made into three groups, according to color of pigment.

I. The B. prodigiosus group, including
B. prodigiosus,
B. ruber indicus,
B. ruber plymouthensis,
B. kiliensis (B. ruber balticus),
B. miniaceus,
B. rutilus (n. sp.),
B. amyloruber (n. sp.),
B. fuchsinus,
B. ruber miquel.

The members of this group develop on agar a pigment in mass, which shows a large percentage of pure red, with varying quantities of orange and sometimes a small amount of blue, which gives a violet tinge, or of black. They are characterized by the fact that the amount of orange diminishes as the cultures age; at the same time there is an addition of black, i. e., the cultures grow darker. This group includes the "carmine", "blood red", and "violet red" cultures.

II. The B. rubricus group, including
B. rubricus,
B. ruber zimmermann,
B. havaniensis,

B. rufus (n. sp.),
B. rosaceus metalloides.

Since these cultures are slower in development, the pigment appears somewhat later than in the organisms of group I. In young cultures a large percentage of orange is present, and the cultures never grow black, but keep the orange red tone. Here are included "orange red", and "yellowish reddish" cultures.

Table I.

		R	O	W	Blue	Blk	V
B. prodigiosus.	Agar slant, 48 hrs. (1,5 % +)	30	70				
	„ „ 5 da. „	75	25	(met. lus.)			
	„ „ 10 „ „	90	10	„	„		
	Potato 48 hrs.	85	15				
	„ 10 da.	60	10			30	(m. l.)
	Gel. stab „ „ (liq.)	90	10				
B. ruber balticus.	Agar slant, 48 hrs. (1,5 % +)	85	15	(met. lus.)			
	„ „ 10 da. „						
	surface	65	35				
	depth	100					
	Potato 48 hrs.	80	15		5		
	„ 6 da.	95	2½		2½		(m. l.)
	Gel. stab. 10 da. (liq.)	90	10				
	„ „ 10 da. (pellicle)	70	30				
B. rutilus (n. sp., 1900)	Agar slant, 24 hrs. (1,5 % +)	50	40	10			
	„ „ 48 „ „	80	20				
	„ „ 10 da. „	68	14		3	15	
	„ „ 10 „	nearly black					
	Gel. stab. 10 „ (liq.)	90	10				
B. ruber miquel.	Agar slant, 48 hrs. (1,5 % +)	80	20	(met. lus.)			
	„ „ 10 da.	85	10			5	(m. l.)
	Potato 48 hrs.	62	26		12		
	„ 10 da.	68	22		10		
	Gel. stab. 10 „ (colony)	80	20				
B. ruber indicus.	Agar slant, 48 hrs. (1,5 % —)	70	25	5			
	„ „ 10 da. „	80	10			10	
	Potato 5 „		30				70
	Gel. stab. 10 „ (pellicle)	75	25				
	„ „ 10 „ (liq.)			100			
B. ruber plymouthensis.	Agar slant, 48 hrs. (1,5 % +)	57	35		8		
	„ „ 10 da.	75	20		5		
	Potato 48 hrs.	30	22	23			25
	„ „ 10 da.	33	35	10			22
	Gel. stab. 10 „ (pellicle)	80	20				
	„ „ 10 „ (liq.)			100			
B. rubricus (n. sp.) (slow)	Agar slant, 10 days	63	30	2	5		
B. havaniensis	„ „ 10 „	55	45				
B. rosaceus metalloides	„ „ 10 „	57	43				
B. mycoides roseus	„ „ 3 „ (1,5 % +)	17	18	65			
	„ „ 5 „	15	35	50			
	„ „ 10 „	42	35	23			
	Potato 10 „ (wrinkled)	57	33	6	4		
B. mycoides coral. (n. sp.)	Agar slant, 10 „ (1,5 % —)	50	25	25			
	Potato 10 „ (smooth)	28	42	18	12		

III. The B. mycoides roseus group, including
B. mycoides roseus,
B. mycoides corallinus (n. sp.),
B. rubropertinctus,
B. latericeus (?).

This group differs from the last in the presence of a considerable percentage of white, with sometimes a trace of yellow. Here would belong the so-called "salmon pink", "coral pink", "rose", or "flesh colored" cultures.

Cultures producing "soluble" red pigment may be classed as the Lactis erythrogenes group, including

B. lactis erythrogenes,
B. rutilescens (n. sp.),
B. rubefaciens,
B. lactorubefaciens.

B. mesentericus ruber is peculiar in producing its pink or red pigment only on potato, not upon agar.

The term "Prodigiosus group" as used below, refers then to the series of Group I as described.

II. Variability in the Prodigiosus group.

1. Introductory.

In the discussion of variability or variation as regards bacteria, several complicating factors are present. In the first place, the facility with which bacteria, more than any other class of organisms, respond and adapt themselves to changes in the nature of their environment is a constant character in their biology. Again, a bacterial type, as we assume it, is more or less artificial, developed and maintained by our methods of culture, which are so arranged as to reduce or eliminate variation. And the old simple distinctions between typical varieties are now complicated not only by the natural variability of the organisms, but by the conception of intermediary paratypes, each with its own possibilities of variation.

The variability of bacteria, whether manifested spontaneously or under compulsion, seems to find its expression principally in the loss of certain characters or in their return after loss. If, for instance, B. anthracis be exposed to a temperature of 42°, B. tetani to a gradual admission of oxygen, or B. prodigiosus to the action of light, the organisms will all live and develop, but one will lose its power of sporulation, another its virulence, the other, its power of pigment production; and unless the abnormal conditions be maintained for many generations, and the process fostered by artificial selection, the organisms will, upon restoration to their usual environment, revert to their original "normal" type. These and many similar observations indicate that bacteria have a certain number of biological characters, without which they may continue to live; that these characters may be lost as a result of environmental modification, but are so far typical that they tend strongly to return.

This "tendency to return" finds its nearest explanation quantitatively, that is, in considering as a factor in variation not only the effect of the environment upon the organism, but also the nature of the compound of characters which we term a type. The aber-

rant or white forms among the red, for example, may be considered as due to sudden or irregular predominance of characters always present, though latent or much in the minority; and the problem before the student of variability is the determination of the "lowest terms" to which an organism can be reduced, the discovery of the minimum and the maximum of characters which can be found to belong to each type.

The only means at our command for such investigation is the modification of the nutritive medium. Such culture media as agar, the composition of which is uncertain, do not give definite results; and I have employed, as set forth below, compounds whose elements and arrangement are more accurately known. Whatever modifications of the organism, i. e., whatever response, by disappearance of characters, has followed change in the environment, I have set down under the general head of range of normal variation, reserving the term discontinuous variation or mutation for the sudden appearance of by-forms without any apparent cause. For such sports I do not offer explanation. They may be due to a phenomenon parallel to what Weismann, speaking of budding, calls "abnormal differential nuclear division", or they may arise in response to physical external causes, confined to a very limited area, and invisible to us. But along with the cases of "discontinuous variation" in the higher organisms, they offer an interesting problem in the origin and differentiation of varieties.

2. Discontinuous variation or mutation.

Variations of bacterial cultures, apparently spontaneous in character, have been frequently noted. Dyar (65) considered that when tubes were filled with media from the same flask, inoculated at the same time from the same culture, and grown on the same shelf, variation resulting in such a series was "discontinuous". The possibility of contamination in bacterial cultures which may thus suddenly vary is always a question, which can only be decided by testing the new variety through a sufficient number of unchanged differential characters, as was done by Dyar in studying a "crusty" variety of B. lactis erythrogenes. The tendency, upon plating such a variety, for some of the colonies to revert to the parent type may be viewed as proof of the true nature of the variation.

Among chromogenic cultures the most frequently observed "sport" variation is in the appearance of colorless colonies upon a plate where the majority of colonies are normal. Such colonies are of course more numerous in plates made from old or degenerate cultures, and decrease gradually with successive transferences. Thus, five B. prodigiosus cultures which were obtained from different laboratories showed the following variations in the early platings. All the plates were made in the same way, and were second dilution. The original agar cultures were evidently young, were all growing well, and were all pink or red in color except

No. V, which showed no pigment, and, as I was informed, had not for at least two months back. The first result on 48 hour agar plate was:

B. prod. IV, 200 small colonies, apparently all orange red.
B. prod. V, 2 pink colonies, 48 white.
B. prod. VI, 300 to 400 colonies, all red.
B. prod. VII, 35 soft spreading white colonies, 3 pink.
B. prod. VIII, 5 smooth round colonies, all violet red.

All the agar streak inoculations from red colonies, and some from white ones, gave ordinary red cultures of varying intensity; and it was evident that the number of abnormal white colonies which appeared as "sports" on the plates were in each case only an indication of the degree to which the colorless variations of the original cultures had gained an ascendency over the red type through continued unfavorable conditions, probably because of long intervals between transferences. That this was the case was shown by a second plating after rejuvenation, fifteen days later. The results of this were:

B. prod. IV. 400 brilliant vermilion colonies.
B. prod. V, 50 violet red colonies, 5 white ones.
B. prod. VI, — all red.
B. prod. VII, 683 colonies, some spreading, all vermilion.
B. prod. VIII, 26 smooth violet red colonies, 1 white.

There are, however, exceptions to the good results of rejuvenation, unless plating and careful selection of colonies is a part of the process. It sometimes occurs that an old culture on neutral agar which has stood in the stock case two or three months will give a brillant pigment on the first transference to neutral agar again, while successive inoculations will seem to diminish the pigment production. The first vigor may be the result of natural selection, but I have no explanation to offer for the later deterioriation in this case.

Light colored or colorless colonies which appear as discontinuous variations upon plates often give rise to apparently constant varieties. Such a colony of B. ruber miquel produced a luxuriant white agar streak, which showed only a few pin-point dots of red. This was allowed to grow for several weeks, and the next and several future transfers gave pure white cultures with all the other characters of B. ruber miquel. Davis[1]) notes "sports" of B. rosaceus metalloides which gave rise to dark and to light colored varieties.

Among my series several instances of discontinuous variation in mass cultures have appeared. A "crusty" variety of B. amyloruber occurred, as in the case of Dyar's B. lactis erythrogenes, after a summer's storage. The original culture had not become contaminated, the pigment of the wrinkled crusty culture was identical in violet red color and rapidity of development with that

1) Davis, N. F. (Science, Vol. XIII. 1901. p. 324.)

of the original, and all the other characters were true. Upon plating, some colonies gave rise to the original soft smooth growth; a series of cultures made after exposing the variety for varying lengths of time to the sunlight also showed one tube like the original, a tube made after 30 minutes' exposure. This result is explicable on the theory of the selective action of sunlight, as noted below.

Variations in colony contour, noted in the case of B. ruber indicus, B. rutilus, B. fuchsinus, B. amyloruber, and rarely for B. kiliensis, where proteus-like and round surface colonies appeared on the same plate, are to be explained partly through physical conditions of the media, and partly by spontaneous variations which arise in the viscidity of the capsular envelope or in the motility of the organism. Agar streaks from the proteus colonies were sometimes slightly more spreading, but the next plate might show total reversion to the round type.

B. plymouthensis is recorded in the original description as differing from B. prodigiosus in marked viscosity on agar and potato cultures. Dyar (loc. cit.) uses viscidity of B. plymouthensis to distinguish it from B. prodigiosus and B. rosaceus metalloides. My culture also differed in producing gas in lactose and sucrose bouillon and in standard asparagin dextrose solution, as well as in a strong fecal odor. During a two years' observation of this culture, viscosity seemed as constant a character of B. plymouthensis as any other of these differences. But the next year, upon revival of the cultures after two months' summer storage, B. prodigiosus I was found to evince the agar culture viscosity of B. plymouthensis. Contamination naturally suggested itself as first explanation, but plating and examination showed the prodigiosus culture to be true in all other respects as noted above, and it was necessary to ascribe the viscosity to a variation which had arisen in the old summer culture and become dominant accidentally in the first plating. Three different cultures now show this peculiarity, the third, B. prodigiosus VIII, presenting it when received at the laboratory. These observations make it necessary to drop the character of viscosity or capsule formation as differential for B. plymouthensis.

In general, what we are in the habit of regarding as important biological characters are not subject to sudden or spontaneous variation; i. e., the power of liquefying gelatin, of producing gas, or of coagulating milk, does not appear or disappear abruptly with no apparent cause. As has been observed in varieties appearing in the same culture of B. coli[1]), the variants are chiefly due to a morphological change, such as the production of more or less of the capsular substance upon which often depends the configuration of surface colonies, or to change in an easily disturbed physiological character such as excretion of pigment.

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1) Smith, T., and Reagh, A. L. The agglutination affinities of related bacteria parasitic in different hosts. (Journ. of Med. Research, Vol. IX. 1903, p. 270.)

3. Range of normal variation.

a) Growth and pigment on ordinary culture media. Morphology.

All the cultures of the Prodigiosus group, except B. kiliensis and B. ruber miquel, are small, actively motile bacilli. B. kiliensis is distinctly larger than B. prodigiosus, while B. ruber miquel is larger still and non-motile. None have spores. A gelatinous capsule is often present in B. prodigiosus and B. ruber plymouthensis. All of these organisms tend to be somewhat larger on solid media, especially on potato.

B. prodigiosus and B. kiliensis showed peritrichial flagella; the others were not examined for flagella.

Cultural features.

Gelatin. Plate colonies vary but slightly beyond the differences in appearance due to variation in the viscosity of the medium. In general the colonies are all like those described for B. prodigiosus, with slight variations as to time and manner in which pigment and liquefaction appear. B. ruber miquel only, does not liquefy gelatin. The same variations in time and degree of liquefaction are seen in gelatin tube cultures, even in parallel inoculations made at the same time, into the same lot of gelatin and from the same culture. This is, however, not an unusual variation in a series of tubes inoculated from one colony, and is probably due in part, as shown by Whipple[1]), to slight physical disturbances in the action of the proteolytic enzyme.

Agar. Variation in the agar colonies, principally in contour and in coloration, is due to the same causes as in the gelatin colonies. Proteus-like colonies often occur, i. e., in B. ruber indicus, B. rutilus, B. fuchsinus, and rarely in B. kiliensis, but the usual Prodigiosus form is round. Pigment usually appears in granular masses throughout the colony, but often the colonies show fine concentric rings of pigmentation, with darker or lighter centres. My attempts to make accurate colored reproductions of these proved futile, since they are quite inconstant. One plate of B. prodigiosus IV may be normally and uniformly pigmented; on another all colonies may become red from the edges inward, leaving at first a white centre; other cultures may have violet red centres and white edges. It is noticeable in such variations that they do not occur on the same plate, i. e., they are regular responsive, and not sport variations.

Potato. The tendency to consider potato as a rejuvenating medium for B. prodigiosus is borne out by the vigorous growth and pigmentation of B. prodigiosus I—VIII on this medium, cf. also B. kiliensis, rutilus, amyloruber, and ruber miquel. For B. ruber indicus, B. plymouthensis, and B. miniaceus, however, potato cultures were unsatisfactory for chromogenesis.

Bouillon. Dense turbidity is always produced in bouillon by

1) Whipple, G. C., On the physical properties of gelatine, etc. (Technology Quarterly. Vol. XV. 1903. p. 159.)

all of these cultures, but great variation is shown in the amount of pigment elaborated and in the formation of pellicle. The amount of sugar present in the medium has an effect upon pigment production, as noted in the descriptions of the separate cultures; the reaction of the bouillon is also a feature, more pigment being produced in bouillon of slightly acid reaction. Usually in sugar free neutral bouillon B. prodigiosus I—IV, VI, VII, and B. ruber miquel show slight coloration of the liquid and a red surface ring, but little true pellicle; B. kiliensis and B. prodigiosus V give thick orange red surface membranes, while B. plymouthensis, B. miniaceus, and B. prodigiosus VIII show color only in a pink or violet surface ring, and B. ruber indicus usually lacks pigment entirely in ordinary meat bouillon but produces it in abundance in a peptone solution. On the other hand B. rutilus and B. amyloruber color the entire liquid deep red, in observing which fact we may remember their recent isolation.

Milk. All except B. amyloruber and B. ruber miquel acidify milk in 24 hours and coagulate it in from 24 (B. rutilus) to 72 hours at room temperature. Some of the cultures show peptonization of the casein. B. amyloruber does not coagulate, but precipitates the casein, while B. ruber miquel produces no change in milk except that of red pigmentation. Almost no variation is seen in these reactions in milk.

Gas production. The appended table shows that great variation is evinced in this respect, not only in the group, but in the same organism at different times, with different stocks of bouillon. The table gives only the limits of numerous determinations made with neutral 1,5 % dextrose bouillon.

Table II.

	Dextrose		Lactose		Saccharose	
	Per cent. of Gas	Per cent. of CO_2	Per cent. of Gas	Per cent. of CO_2	Per cent. of Gas	Per cent. of CO_2
B. prodigiosus I, II, III	0—34,5	100	—	—	—	—
„ „ IV	10		—	—	10—20	
„ „ V	70	55	32	20	70	50
„ „ VI	40	98	—	—	40	98
„ „ VII	10—20		—	—	10—20	
„ „ VIII	—	—	—	—	—	—
B. ruber indicus I, II	30—70	100	—	—	20—25	100
B. kiliensis (B. r. balt.)	30—40	26—28	27	21	30	20
B. plymouthensis I, II	70—78	70—78	38—42	70—72	25—30	70—75
B. miniaceus I, II, III	40—60	40—65	36—40	35—57	30—53	20—70
B. rutilus (n. sp.)	28—91	100—65	—	—	6—88	100—60
B. ruber miquel	34—46	77—79	—	—	—	—
B. amyloruber (n. sp) and B. fuchsinus	—	—	—	—	—	—

It is to be remarked that, except in the case of B. rutilus, the relation of CO_2 to the total gas produced remains fairly constant for each organism. B. rutilus produced 60—65 % CO_2 at first, but with gradual loss, after isolation, of the power to produce

a large amount of gas, it seemed also to lose the power to form anything but CO_2. B. prodigiosus I usually failed to produce gas in 1% dextrose, 1,5—2 % being more favorable.

Oxygen and temperature relations. All of this group are facultative anaerobes, but grow without pigment in the absence of oxygen. Only B. kiliensis and B. ruber indicus produce pigment at 37° C, although all are able to grow at that temperature.

Indol production, nitrate reduction, odor. No indol is formed by the members of this group. Nitrate is reduced to nitrite in each case and often to free gas. The trimethylamine odor is often present in cultures of the Prodigiosus group, and a strong fecal odor is characteristic of B. plymouthensis, B. prodigiosus V, and VIII.

B. ruber indicus only is said to be pathogenic for laboratory animals. 2 ccm of a 48 hour bouillon culture inoculated intraperitoneally killed a mouse in 48 hours. Unfortunately I was unable to make an autopsy. 20 ccm of a 48 hour agar suspension failed to produce any effect upon a guinea pig[1]).

b) Growth and pigment on special solid media.

The earlier investigators of red chromogenic bacteria, Ehrenberg (26), Fresenius (27), and Cohn (30), concerned themselves chiefly with the systematic position of B. prodigiosus; Erdmann (28), and Schroeter (29) worked with the chemical nature of the pigment; Schottelius (35) was the first to pursue ecological studies upon the pigment production, without, however, much attention to the composition of the cultural media. His conclusions as to the conditions necessary for pigmentation were 1) a sufficient supply of atmospheric air, 2) a suitable temperature. Wasserzug (37) experimented upon the effect of alkaline and of acid media, obtaining colorless races in alkaline bouillon. Kuebler (38) repeated Wasserzug's procedure, but contradicted him and confirmed Schottelius in asserting the non-permanency of white cultures obtained by high temperature and alkaline media.

The next important paper on the subject was that of Galeotti (15), who studied eight chromogenic organisms, among them B. prodigiosus, Lustig's "red bacillus", and B. lactis erythrogenes. He found that B. prodigiosus gave less pigment in liquid media than in solid, but that this was not due, as Wasserzug had thought, to lack of oxygen, since an atmosphere of pure oxygen produced no better chromogenesis. He decreased the amount of peptone in the agar, and inferred that a scarcity of proteid did not prevent pigment production. B. prodigiosus was the only organism of his series in which pigment production could be impeded

1) Subcutaneous inoculation of 2 ccm. 48 hr. agar slant culture, suspended in 5ccm 0,85 % Nacl solution, causes illness in rabbits. A large abscess forms at the site of inoculation, from which a vigorous and pigmentforming culture of B. ruber indicus was isolated at the end of two weeks. Abscesses are also formed by B. prodigiosus I and VII, B. rutilus (n. sp.), and B. amyloruber (n. sp.). Further investigations upon the pathogenicity as well as upon the agglutinative properties of these organisms are in progress.

by a high temperature without interfering with the luxuriance of development. White light had a limiting, red light very little effect upon the pigment; lack of oxygen and also pure oxygen were both detrimental[1]). Galeotti thus concluded 1) that the power of chromogenesis in bacteria is not connected indissolubly with the life of those bacteria; such microorganisms may be able to live without producing their characteristic pigment. 2) that the conditions of life which affect the chromogenic power are generally those which have an unfavorable influence upon the bacteria themselves in all their functions. 3) that, given conditions unfavorable to the production of pigment by any special chromogenic microorganism, that organism will, in a longer or shorter period of time, reacquire the power of pigment production by adapting itself to the unfavorable conditions.

Galeotti's first conclusion is supported by most investigators of bacterial chromogenesis, and by all the observations which are here cited as to the ease with which the power of pigment production is lost by some microorganisms. His second and third generalizations are, however, debatable.

Noesske (18) says, speaking of B. pyocyaneus and B. prodigiosus, — "Nicht trotz, sondern infolge zu üppiger Vegetationsbedingungen sistiert manchmal die Farbstoffbildung auf unserer gebräuchlichen Bouillon." Noesske is supported by Woolley (21), who concludes that B. pyocyaneus, B. janthinus, and B. prodigiosus show better development but less pigment in sugar media as compared with sugar free media; pigment is produced more easily in 1 % than in 2 % sugar media, with the exception of B. prodigiosus, which is alike in both cases, but better in glucose than in lactose or saccharose.

The presence of sugar in nutritive media was, according to Wasserzug, detrimental to the pigment production of B. prodigiosus; but Laurent (57) found that the influence of sugar could be counteracted by the addition of alkali, i. e., that the injurious effect was only indirect, through the acid formed from the sugar by the bacteria.

Although no very definite conclusions can be drawn from media containing so many unknown elements as our ordinary bouillon or agar, a few preliminary experiments were made with agar as to the effect following on the elimination of some of its constituents. Cultures which had been 4 months on neutral sugar-free agar were transferred to similar fresh agar slant tubes. After rejuvenation by the bouillon, gelatin, and agar plate method, sugar-free and 1 %

1) cf. Pfeffer, W. Ber. der Kgl. Sächs. Gesellsch. d. Wissensch. Leipzig, math.-phys. Klasse. 1896, p. 379, ref. Baumgartens Jahresb. 1896, p. 705. „Farbstoffbildende Bakterien vermögen den Sauerstoff locker zu binden (ähnlich wie das Hämoglobin) und ihn im sauerstofffreien Raum wieder abzugeben. Der Träger dieser Erscheinung ist der Farbstoff, der die gleiche Wirkung auch isoliert im alkoholischen Extrakt zeigt, während bei farblosen Bakterien ein Gleiches noch nie beobachtet ist. Die Farbstoffbildung, die bisher mehr als eine Luxusproduktion erscheint, erscheint hiernach vielleicht in einem für die Art ungleich zweckmässigeren Sinne, indem sie vielleicht die Bedeutung hat, dem betr. Bakterium eine stets bereite Sauerstoffreserve zu sichern."

dextrose agar slant tubes were inoculated from the same colonies, with the following results: —

Table III.

	5 days, Sugar-free neutral agar, after 4 months on neutr. agar		Rejuvenated							
			24 hrs.		24 hrs.		5 days		5 days	
			Sugar-free agar		Glucose agar		Sugar-free agar		Glucose agar	
	Gr.	Pigm.	Gr.	Pigm.	Gr.	Pigm.	Gr.	Pigm.	Gr.	Pigm.
B. prodigiosus I	lux.	red	lux.	deep pink	lux.	pink, trace	lux.	red	lux.	red and white
B. ruber indicus I	„	pink	„	„	„	—	„	pink, trace	„	pink and white
B. „ „ II	„	„	„	„	„	—	„	pink, trace	„	pink and white
B. ruber balticus	„	orange red	„	„	„	pink, trace	„	orange red	„	red at top [1])
B. ruber plym. I	„	pink	„	pink trace	„	red, luster,	thin	red, trace	„	violet red, lustre
B. ruber miquel	„	red	thin	orange red	„	pink	„	orange red	„	violet red
B. rutilus (n. sp.)	„	pink	„	red	„	„	„	red	„	red and white
B. amyloruber (n. sp.)	wrinkled	violet red	„	deep pink	„	—	„	violet red	„	dark violet red
B. plym. II	lux.	pink	„	—	„	red, luster	„	—	„	red, luster
B. kiliensis	„	red, trace	„	—	„	red, trace	fair	—	„	red, trace
B. miniaceus	„		„	pink trace		red, luster	thin	pink, trace	„	red, luster

A comparison of the five-day sugar-free agar growth shows B. plymouthensis I and B. rutilus to have been apparently greatly benefited by the rejuvenating process, B. kiliensis and B. plymouthensis II as adversely affected, and the others as not affected at all. This may be due in part to the fact that no particular attempt was made to wait for colonies showing the highest pigmentation, but only to inoculate both tubes from the same colony.

When we compare the results of 24 hours for the two agars, we find some interesting differences; the cultures are divisible at once into two classes. B. prodigiosus I, B. ruber indicus I and II, B. ruber balticus, B. ruber miquel, B. rutilus, and B. amyloruber, all show more pigment on sugar free agar. With the exception of B. ruber miquel, which was thin on sugar free agar, the development was of about the same degree of luxuriance on all. Three other cultures, B. plymouthensis I and II and B. miniaceus, gave a surprising result in comparison with the others, — maximum pigmentation with green luster upon the sugar medium,

1) Later, more luxuriant and darker pigment on glucose agar.

and very little or none at all upon the sugar free agar. B. kiliensis is also of interest; it is a degenerate culture of feeble pigmentation, and still shows a lingering tendency to return to normal if opportunity affords, i. e., upon a fresh transfer from an old culture, or upon transference to a new (here a sugar) medium.

The five day growth presents the same differences, though less distincfly. The mosaic appearance of B. ruber indicus and B. rutilus is a beautiful expression of the existence of some pigment-producing individuals among the mass of those not producing pigment. This seems to argue that, here on solid media at least, the presence of sugar has the effect not of modifying the pigment, but of either permitting it unmodified or of inhibiting it. That the acid formed from the sugar is the inhibiting influence may be questioned because of the early appearance of the differentiation. The effect of acid directly upon the pigment may explain the more violet red color of B. ruber miquel after five days, and the darker color of B. amyloruber. The late and luxuriant appearance of pigment in B. ruber balticus on sugar agar is a peculiar result entirely inexplicable on the acid theory. It is to be noted that the three cultures which produce early luxuriant pigment on sugar agar are among those, of the whole series, which produce the most active fermentation of sugars in the fermentation tube.

It seemed possible to obtain some light upon the question of growth-luxuriance and pigment-luxuriance by eliminating the bouillon from the ordinary agar medium. Accordingly a series was grown upon agar 1,5 %, peptone 1 %, and water. This medium was first tried of different reactions, 1,5 % acid, neutral, and 1,5 % alkaline to phenolphthalein. The same medium, neutral, was used with the addition severally of 1 % pure dextrose, lactose, and saccharose. Two differences between the "meat-free" medium and the ordinary nutrient agar were noticeable, though hardly measurable. The growth is more limited, i. e., less spreading and "massy", and the pigment is in general more intense in the former. Some of this depends, without doubt, upon the vigor of the culture, a particularly vigorous strain being able to overcome slight differences in the media, expressed, in feeble strains like B. kiliensis, by a series showing distinct gradation.

(See Table IV p. 33.)

This 48 hour table brings out several points:

1) The tendency to violet red pigment on the more acid, to orange red on the more alkaline media.

2) The similarity of pigment color on dextrose and saccharose agar to that on sugar free agar of acid reaction. That is, acid is probably formed from assimilation of these two sugars.

3) The similarity of pigment color on lactose agar to that formed on sugar free neutral agar; i. e., lactose is probably not easily assimilated.

This and the last named point seem to afford evidence of the activity of sucrase but not of lactase.

Table IV.

48 hours.	Agar 1,5 %, Peptone 1 %						Agar 1,5 %, Peptone 1 %					
	1,5 % +		O		1,5 % —		1 % Dex.		1 % Lact.		1 % Sacch.	
	Gr.	Pigm.	Gr.	Pigm.	Gr.	Pigm.	Gr.	Pigm.	Gr.	Pigm.	Gr.	Pigm.
B. prod. I	++	violet red +	+	violet red +	sl.	—	+	violet red +	+	red +	+	violet red +
B. „ II	++	violet red +	+	violet red +	sl.	—	++	violet red +	+	orange red +	+	violet red +
B. „ III	++	violet red +	+	violet red +	sl.	—	++	violet red +	+	orange red +	+	violet red +
B. „ IV	++	red ++	++	vermilion ++	++	vermilion +	++	dark red ++ luster	++	orange red ++ luster	++	dark red ++ luster
B. „ V	++	red ++	+	red +	sl.	sl.	+	violet red +	+	orange red +	+	violet red +
B. „ VI	++	violet red ++ luster	+	red +	+	red +	+	violet red +	++	orange red ++	++	dark violet ++ red lustre
B. „ VII	++	vermilion ++	++	vermilion ++	++	vermilion ++	++	violet red ++ lustre	++	orange red ++	++	violet red ++ lustre
B. „ VIII	+	violet red +	+	violet red +	+	violet red +	+	violet +	+	violet +	+	violet +
B. r. ind. I	+ dry	orange red +	++ dry	orange red +	+ dry	orange red +	+ dry	dark red +	+ dry	orange red +	+ dry	orange red +
B. „ „ II	++ dry	orange red ++ luster	+ dry	orange red +	+ dry	pale orange red	+	dark red ++	+	orange red ++	+	orange red ++
B. r. balt.	++	orange red ++	+	orange red +	+	—	++	violet red ++ luster	+	orange red +	++	dark red ++
B. r. miquel	+	red +	sl.	—	+	—	+	dark red ++	+	—	+	dark red +
B. r. ply. I	sl.	—	sl.	—	sl.	—	++	dark red ++ luster	+	pink	++	dark red ++ luster
B. rutilus	++	violet red ++	+	red +	sl. +	orange red +	+	—	+	orange red +	+	violet red +
B. amylo-ruber	++	violet red ++	++	red +	+	orange red sl.	sl.	pale violet	+	violet red +	++	violet red ++
B. ply. II	++	—	+	—	+	—	++	dots red	++	—	++	dots red
B. kiliensis	+	deep pink +	+	pale pink +	+	—	++	dark red ++	++	—	++	pink

4) The orange red color of B. ruber indicus I and II on acid agar is probably due to alkali-production. Query: — Is the large amount of acid formed by B. ruber indicus in bouillon and on ordinary meat agar, as evinced by its better growth on alkaline meat agar. a result of the meat albumins?

5) The range of color in B. amyloruber, as follows:

	Red	Orange	Blue
1,5 % acid	87	10	3
Saccharose	70	20	10
Dextrose	50	30	20
Neutral and Lactose	65	15	20
1,5 % alkaline	45	55	

6) the inhibition of pigment by dextrose in the case of B. rutilus, although the saccharose tube shows evidence of assimilation, i. e., is like 1,5 % acid.

After five days' growth some of these cultures were slightly less characteristic, as would naturally result from the constantly increasing complexity of the metabolic products. For example, B. prodigiosus IV had become vermilion on 1,5 % acid agar, no doubt as a result of the alkali produced by the vigorously growing culture. Other of the more slowly developing cultures bore out the results of the first 48 hours, e. g., B. prodigiosus I—IV had produced orange red pigment on 1,5 % alkaline agar, and B. plymouthensis I slight violet red color on acid agar. Metallic green luster had appeared for several cultures, noticeably for B. ruber balticus on 1,5 % acid agar, and for B. plymouthensis I and II on dextrose and on saccharose agar. Orange red color had changed to red on lactose agar for B. prodigiosus V and for B. rutilus, but excepting these and the colorless lactose agar cultures of B. ruber miquel and B. plym. II, the orange tone still held for the series on this medium, i. e., the alkali produced by the bacteria was not neutralized by acid formed from the sugar. It would seem that since B. ruber balticus, B. plymouthensis I, and B. miniaceus ferment lactose with gas production, these cultures would show a decrease of the orange tone.

Although the relative results arrived at on the above media are of some value, their absolute value is lessened because of the unknown factors present in agar and peptone. Agar itself contains a certain amount of carbohydrate, galactose (Bauer, Jour. Prakt. Chemie, B. XXX. p. 367), which is probably assimilable by the organisms. A further reduction of the medium, by leaving out the peptone, resulted in very feeble white growth in five days. Three cultures only showed a trace of pigment, — B. ruber indicus II, B. rutilus, and B. prodigiosus VII. The last named showed a green iridescence on the thin pink layer.

In the endeavor to do away with agar and still have a solid medium for pigment production, the following mixtures were employed, and gave interesting comparative results. The flour and starch were cooked, poured into Petri dishes, sterilized by discontinuous method, and inoculated.

Table V.

	Rice flour 35 %, Pept. 1 %, water		Starch 10 %, Pept. 1 %, water		Starch 10%, water	
	Gr.	Pigm.	Gr.	Pigm.	Gr.	Pigm.
B. prodigiosus I	good	red	good	red	slight	slight, pink
B. ruber balticus	fair	dark red	luxuriant spreading	intense red luster	thin spreading	pink
B. kiliensis	good	red	good	red	„	pink
B. ruber miquel	fair	red	luxuriant	violet red	slight	red
B. rutilus (n. sp.)	good	dark red	„	cream white	—	—
B. amyloruber (n. sp.)	good	violet red	luxuriant spreading	intense violet red luster	spreading	pink

Rice flour, which gave luxuriant growth when used with bouillon by Schneider (19) and Petrow (58), gives good pigment, but rather a thin dry layer, when bouillon is omitted.

The similarity between B. ruber balticus and B. kiliensis was brought out by their development on starch and water. On the other hand B. prodigiosus, B. ruber miquel, and B. rutilus scarcely developed at all on cooked starch, but with the addition of 1 % peptone gave a luxuriant growth. B. rutilus was peculiar here in its absence of pigment, while B. amyloruber for the first time produced, in addition to its usual vivid dark red color, a distinct green metallic luster. The development of this organism was evidently at its height of luxuriance on the starch-peptone medium. Considerable liquefaction of the solid starch also took place, an evidence of the production of a diastatic ferment by B. amyloruber. After two weeks the semi-solid mass on this plate was rubbed up in distilled water, filtered germ free and tested. 12 ccm peptonized 5 ccm of 10 % gelatin and water in two hours at 37° C. A half inch cube of 10 % cooked starch was not liquefied by the filtrate in 24 hours at 37° C, and I am unable to adduce any evidence, other than that of the first observation, for the presence of diastase. Fermi (22), and Gorini (41) report negative results as regards diastatic ferments from B. prodigiosus and "B. ruber".

c. Growth and pigment in non-proteid media.

The value of the above experiments as to the affect of sugars could only be tested by their repetition with non-proteid media, where all the elements which go to make up the nutritive supply of the organisms are definitely known. The only investigations of this sort hitherto undertaken for red chromogenic forms have been upon B. prodigiosus and B. kiliensis. Those upon the latter organism, by Laurent (57), were carried out mainly to test the effect of acid in the medium, and led Laurent to the conclusion that an alkaline reaction was most favorable to growth and pigmentation. But

since in every case Laurent used media which contained either saccharose or glycerine, and in which the initial alkaline reaction had only the effect of neutralizing the acid produced by the organism from these substances, his results prove nothing definite as to the reaction best for non-fermentable media. As, also, the results of Kuntze (46), Noesske (18), Luckhardt (16), and Sullivan (20) upon B. prodigiosus in non-proteid media have disagreed, the following tables of experiments on the Prodigiosus series will be of interest[1]).

Table VI.

	Sol. A. Asp. 0,2 % $MgSO_4$ 0,1 % K_2HPO_4 0,1 %		Sol. B. Asp. 0,2 % K_2HPO_4 0,1 %		Sol. C. Asp. 0,2 % $MgSO_4$		Sol. D. Asp. 0,2 %		Sol. E. Asp. 1,0 % $MgSO_4$ 0,1 % K_2HPO_4 0,1 %		Sol. F. Asp. 0,2 % $MgSO_4$ 0,1 % K_2HPO_4 0,1 % Glycerine 2,0 %	
	Gr.	Pigm.	Gr	Pigm.	Gr.	Pigm.	Gr.	Pigm.	Gr.	Pigm.	Gr.	Pigm.
B. prod. I	++	—	+	—	—	—	—	—	++	5 da. tr.	15 days ++	—
B. „ II	++	—	+	—	—	—	—	—	++	5 da. tr.	++	—
B. „ III	++	—	+	—	—	—	—	—	++	5 da. tr.	++	—
B. „ IV	++	—	+	—	—	—	—	—	++	—	++	—
B. „ V	++	—	+	—			—	—	++	—	++	—
B. „ VI	++	—	+	—			—	—	++	—	++	—
B. „ VII	++	48 hrs. + 3 da.++	+	48 hrs. +			—	—	++	48 hrs. + 3 da.++	++	—
B. „ VIII	++	—	+	—			—	—	+	6 da. tr.	++	—
B. rub. ind. I	+	48 hrs. + 3 da.++	+	++	+	++	+tr.	++	++	—	++	—
B. „ „ II	+	48 hrs. + 3 da.++	+	++	+	++	+tr.	++	+	48 hrs. +	++	—
B. rub. balt.	++	—	+	—	—	—	—	—	++	3 da. +	++	—
B. „ plym. I	++	—	+	—	+sl.	—	—	—	++	—	++	sl.
B. „ miquel	++	+	+	—	—	—	—	—	+	+ sl.	++	—
B. rutil. (n. sp.)	+	—	+	—	+sl.	—	—	—	++	—	++	—
B. amylo-ruber (n. sp.)	+	15 days ++	—	—	+sl.	—	+?	—	sl.	15 days +	+	—
B. plym. II	++	—	+	—	—	—	—	—	++	—	+	—

1) The water used in these solutions was redistilled in glass. The inoculations were made in flasks of Jena glass previously cleaned in acid and rinsed repeatedly in distilled water. The reaction was neutral to phenolphthalein.

These series bring out interesting results in the behavior of the cultures. In standard asparagin solution (Sol. A), B. prodigiosus I, tested five times, and B. prod. II—VI and VIII, tested twice, grew luxuriantly, i. e., showed dense white cloudiness, but showed no trace of pigment. B. ruber balticus, B. plymouthensis I and II and B. rutilus gave the same results, except that B. rutilus developed less cloudiness. On the other hand B. prod. VII, B. ruber indicus I and II, B. ruber miquel, and B. amyloruber produced a good red coloration of the medium, but none grew luxuriantly. except B. ruber miquel and B. prod. VII.

In the next series (Sol. B), $MgSO_4$ was eliminated from the medium in an attempt to determine whether this substance be necessary for the elaboration of red bacterial pigment, as has been shown for the fluorescent pigment. Nearly all the cultures developed, although less readily than in Sol. A; three of them, B. ruber indicus I and II, and B. prodigiosus VII, although showing scarcely any cloudiness, colored the solution a beautiful red in 48 hours. With these three cultures which gave pigment in the absence of $MgSO_4$ further tests were made. A pure 0,2 % solution of asparagin was prepared, and flasks were inoculated by touching the surface of an agar plate colony with a fine needle, or from a growth in Sol. B. In each case B. ruber indicus I and II produced no distinguishable cloudiness of the solution, but slowly and gradually colored it as deep a red as they did the standard solution. B. prodigiosus VII failed to show pigment here, and control cultures of B. ruber balticus and B. ruber miquel also remained perfectly clear and colorless.

These results point toward one of two conclusions. Either the pigment of some cultures of what is here designated as the Prodigiosus group has a different chemical basis from that of others of the group, or on the other hand, great variation occurs among these cultures in their ability to elaborate the same pigment out of the same synthetic material. The results of chemical and spectroscopic analysis of the pigments of B. prodigiosus, B. ruber balticus, and B. ruber indicus (19), (45), (49) give us no reason to believe that they are essentially different. Further, the fact that some cultures do not produce pigment in a 0,2 % asparagin solution even in the presence of $MgSO_4$, while others beside B. ruber indicus have this power, indicates a continuous, rather than a discontinuous variation of the ability to elaborate pigment. Whatever the cause of this may be, it appears from my results to the present time that the different strains or varieties are very constant in their ability or non-ability to form pigment in the above solutions, whether the latter be inoculated from young or old cultures on various media.

In his work on B. prodigiosus, Kuntze used 1—2 % asparagin and 0,1—0,2 % K_2HPO_4, obtaining pigment if $MgSO_4$ were added in smallest crystals. In order to determine whether lack of pigmentation in my series was due to an insuffiency of the organic compound, I increased the asparagin content to 1 %. The first trial

added B. ruber balticus only to the list of color producers. A second set of flasks was inoculated from five day potato cultures, with the result that B. prod. I—III developed a slight trace of pigment. Growth was most luxuriant for all the cultures in this solution, but on the other hand B. ruber indicus I lost its power of producing color by the concentration of the medium.

Standard asparagin solution with the addition of 2,0 % glycerine allowed luxuriant growth, but of the whole series B. plymouthensis I only showed a slight trace of pigment.

It seems evident from these experiments that the conclusions drawn by Kuntze and Noesske regarding the necessity of $MgSO_4$ for pigment formation, and of phosphorus for growth, in the case of B. prodigiosus, do not hold for all of the various strains going under this name. Out of eight strains three did not form pigment even under these conditions, although all were cultures in the height of vigor; one culture produced pigment in the absence of one, and two strains of a closely related form in the absence of both the above substances.

The more luxuriant development of some of the non-pigmented cultures in comparison with the slighter growth of other colored ones, notably B. ruber indicus, seems to confirm the statement made by Noesske and quoted previously in this paper. Further light upon the subject of correlation of chromogenesis and growth, as well as upon the effect of the presence of carbohydrate in the nutritive medium, was obtained by the following experiments.

Table VII.

	Sol. H. Asp. 0,2 % $MgSO_4$ 0,1 % K_2HPO_4 0,1 % Dextrose 1,0 %		Sol. I Asp. 0,2 % $MgSO_4$ 0,1 % K_2HPO_4 0,1 % Lactose 1,0 %		Sol. K Asp. 0,2 % $MgSO_4$ 0,1 % K_2HPO_4 0,1 % Saccharose 1,0 %	
	Gr.	Pigm.	Gr.	Pigm.	Gr.	Pigm.
B. prod. I.	++	tr. 4 da.	++	—	++	—
B. prod. II	++	tr. 8 da.	++	—	++	—
B. prod. III	++	tr. 3 da.	++	—	++	tr. 3 da.
B. prod. IV	++	— 4 da. + 15 da.	++	—	++	—
B. prod. V	++	+ tr. 3 da. tr. 15 da.	++	—	++	+ tr. 3 da.
B. prod. VI	++	tr. 8 da.	++	—	++	+ 8 da.
B. prod. VII	++	+ tr. 4 da. ++ 15 da	++	+ 3 da. + tr. 15 da.	++	+ 4 da. ++ 15 da.
B. prod. VIII	++	+ tr. 4 da	++	—	++	+ tr. 8 da.
B. rub. ind. I	++	—	+	++	++	—
B. rub. ind. II	++	+ 8 da.	+	++	++	—
B. rub. balt.	++	+ 3 da.	++	—	++	+ tr.
B. rub. ply. I	++	+	sl.	—	++	+
B. rub. miquel	++	+ 3 da.	sl.	+ 4 da. ++ 8 da.	++	+
B. rutilus (n. sp.)	++	+ 8 da.	+	—	++	—
B. amyloruber (n. sp.)	++	+	+	+	++	—
B. plym. II	++	—	++	—	++	—

When this table is compared with Table VI the similarity of results with Sol. E and Sol. H is at once noticeable, the presence of dextrose producing much the same effect as the concentration of the medium by increasing the asparagin content. With the addition of dextrose to a standard asparagin solution, growth is luxuriant in all cases, but although there is an increase of pigment production over Sol. A, where in most cases there was none at all, still the amount of pigment for B. prod. I—VI and VIII is at most only a trace. On the other hand, B. prod. VII shows a development of pigment, which at the end of fifteen days is as strong as in the standard solution in three days; and B. ruber indicus I and II show, as in Sol. E, more luxuriant growth with a lessening of pigment production.

Comparing the effect of the different carbohydrates, we see that saccharose behaves on the whole like dextrose, although in some cases the pigment failed to appear in Sol. K, where it did appear in Sol. H. Lactose gives quite different results. In fact, Sol. I containing lactose behaves exactly like Sol. A with no carbohydrates, i. e. pigment is produced with B. prod. VII, B. ruber indicus I and II, B. ruber miquel and B. amyloruber only, these, with the exception of B. prod. VII, showing less luxuriant growth than the rest of the series. This seems an exceedingly interesting result, and falls in line with the conclusion drawn from Table IV, where the color of the pigment gave evidence of the peculiar lack of effect of the presence of lactose.

It is difficult, even here, to arrive at any general conclusion in regard to the relation of growth luxuriance and pigment luxuriance. As has just been stated, the majority of the cultures showing pigment in Sol. I have grown less luxuriantly than the others. The same thing was true with Sol. A. Again, B. ruber indicus I and II tended to lose entirely their power to form pigment when the growth luxuriance was increased by concentrating the medium or by adding glycerine or sugar.

These facts, taken in connection with that of less massy growth and more vivid pigment on peptone agar as against the more complex meat peptone agar, seem to argue in confirmation of Noesske's and Woolley's views. On the other hand, Sol. H, in which dextrose induces luxuriant growth, shows pigment, though only traces of it, in fourteen cases, against four cases in Sol. A without dextrose. It may be that here the sugar contributes chemical or physiological aid to pigment formation as well as to vegetative luxuriance. The contrary effect of glycerine points to this conclusion, since here we have luxuriant growth without pigment.

In this connection also, some of the atypical cultures which have virtually lost the power of forming pigment are interesting. Both B. fuchsinus and B. miniaceus III are among the most vigorous strains of the series in rapidity and amount of development. The latter and B. kiliensis, although colorless, have lost none of their vigor in the fermentation of sugar to gas formation, or in the

liquefaction of gelatin or coagulation of milk. These facts do not support, then, Galeotti's second conclusion, that the conditions of life which affect the chromogenic power are generally those which have an unfavorable influence upon the bacteria themselves in all their functions.

d) The effect of light upon pigment production.

Following the methods of Buchner and of Marshall Ward, Dieudonné (13) confirmed the simpler experiments of Galeotti (15) regarding the effect of light upon bacterial chromogenesis. He used B. prodigiosus and B. fluorescens, and found that the direct sunlight of March, July, and August hindered development in half an hour, while 48 hours of exposure entirely prevented pigmentation and trimethylamine production. B. prodigiosus also liquefied gelatin more feebly. Eleven hours of incandescent electric light killed both organisms. According to Dieudonné's investigations, these effects were produced by the light and not by the heat rays, the violet and ultra violet rays being the destructive agents, and the red and yellow rays having no effect. The injury is chiefly to the germs themselves, the chemical change produced in the medium being a very small factor. Similar results were obtained with B. coli, B. typhosus, and B. anthracis, confirming those of Ward, who, however, limits the injurious effect to the violet end of the blue, the actinic rays.

Beck and Schultz (14) criticised Dieudonné's methods, and observed no injury to the development of B. prodigiosus, B. pyocyaneus etc., from exposure to colored light, though in some cases there was an influence upon chromogenesis (2—3 days). Diffuse daylight was beneficial, darkness sometimes proving injurious, (Staphyl. pyog. aureus and B. fluorescens). Direct sunlight (3 days) produced colorless cultures.

The results of Beck and Schultz do not seem very conclusive; the non-effect of the colored light may have been due to the slight intensity of the rays which passed through their light filters. In a recent paper Oliver (17) used colored glass plates.

A simple comparative study as to the effect of direct sunlight upon a series of my organisms gave the following results. One loop-ful-inoculations were made upon slant agar from 18 hour bouillon cultures which had been grown in the dark; these were used as controls. The cloudy bouillon cultures were then exposed to the March sunlight (according to Dieudonné as effective as that of July), and similar agar slant cultures inoculated at intervals. These agar cultures were then grown in the dark and examined after 24 hours, 48 hours, and ten days. The results are appended.

Table VIII.

	24 hours' growth after exposure to sun of							
	0 min.		5 min.		15 min.		30 min.	
	Gr.	Pigm.	Gr.	Pigm.	Gr.	Pigm.	Gr.	Pigm.
B. prod. I	++	—	+	—	++	—	++	—
B. ruber balt.	++	—	+	—	++	—	+++	—
B. ruber miq.	++	—	dotted +	—	dotted ++	—	dotted +	—
B. rutilus (n. sp.)	++	—	++	—	++	—	+	—
B. amylor. (n. sp.)	++	—	+	—	++	—	+	—
B. rosac. metall.	+	sl.	—	—	—	—	—	—
	48 hours' growth after exposure to sun							
B. prod. I	++	++ red	+	pink	+	pink	+	pink
B. ruber balt.	++	++ red	++	pink	++	+ pink	++	pink
B. ruber miq.	++	++ red	++	orange red	++	orange red	++	pink
B. rutilus (n. sp.)	++	++ red	++	+ slight red	++	pink	++	pink
B. amylor. (n. sp.)	++	++ red	++	++ red	++	+++ red	++	+ red
B. rosac. metall.	++	orange	+	—	+	—	+	—
	10 days' growth after exposure to sun							
B. prod. I	++	++ red	thin	pink	thin	piuk	thin	pink
B. ruber balt.	++	++ red	thin	pink	thin	pink	thin	pink
B. ruber miq.	++	++ red	++	++ red	++	+ red	++	+ dull red
B. rutilus (n. sp.)	++	++ red	thin	pink	thin	pink	thin	pink
B. amylor. (n. sp.)	++	++ dark red	++	++ dark red	++	++ dark red	++	++ dark red
B. rosac. metall.	++	++ orange	++	++ orange	+	+	thin	pale

48 hours after 2 hours exposure to sun.

B. prod. I	Growth normal,	pigment faint pink
B. ruber balt.	„ thin,	„ faint pink
B. ruber miq.	„ very slight,	„ slight
B. rutilus (n. sp.)	„ normal,	„ faint pink
B. amylor. (n. sp.)	„ thinner	„ normal
B. rosac. metall.	no development	

From the tables it will be seen that the 24 hour cultures presented a curious developmental result. The cultures of B. ruber balticus which had been made after 30 minutes' exposure to the sun showed more development than the control, while the other exposures exhibited a comparative decrease of effect, a 5 minutes' exposure giving less growth than one of 15 minutes. Greater development of a 15 minutes' exposure as compared with one of 5 minutes was also shown by B. prodigiosus, B. ruber miquel, and B. amyloruber, so that the possibility of an accidental mechanical error, e. g., the transference of a loopful of germs crowded at one side of the tube by heliotaxis, was excluded from consideration. The experiment was repeated for B. ruber balticus, B. rutilus, and B. amyloruber with the same results.

A possible explanation of the phenomenon may be suggested. Gotschlich (5) has remarked that brief exposure of a culture to an injurious influence may react beneficially to the culture as a whole, by cutting out the weaker organisms, and leaving only the "Ausnahmezellen"; that is, that there may be selective death-rate. On this

supposition the first effect of the sunlight was the destruction of a great number of the less resistant organisms, which accounts for the slighter mass-development of the cultures after the 5 minute exposure. For the remaining and more resistant cells we must then assume that the actinic effect of a 15 minute exposure was stimulating, promoting cell division, somewhat as exposure to increased osmotic pressure[1]) or to lack of oxygen, to heat, etc., induces it in unfertilized parthogenetic eggs. As the eggs in these experiments must be exposed only briefly and then returned to their normal environment if maximal results are to be obtained, so with the bacterial cells. The accelerating effect of the sunlight on growth does not seem to be an enduring one, for later observations upon the same cultures show that development is in reality permanently hindered; as much, in the case of B. prodigiosus, B. ruber balticus, and B. rutilus, by 5 minutes' exposure as by one of 30 minutes, and nearly as much by 5 minutes as by one of 2 hours.

The pigmentation of B. amyloruber and B. ruber balticus also showed, in the 48 hour agar culture, a distinctly better color after the 15 minute exposure. The chromogenesis of the others of the series was markedly decreased by a 5 minute exposure, and only B. ruber miquel showed any recovery after ten days. The greater resistance of B. amyloruber, which was as brilliantly pigmented after two hours' exposure to the sun as at first, was probably due to its recent isolation from river water.

No attempt was made to determine the further history of these cultures.

4. Summary.

The experimental and comparative work done on these cultures of pigment bacteria may be roughly summarized as follows:

1) Notwithstanding the occasional loss of power of pigment production by a previously chromogenic organism, the character of the pigment is markedly constant among red chromogenic bacteria. By constancy is here understood the appearance of pigment of definite color upon nutritive media of known composition and under defined environmental conditions.

2) A collection of about forty red cultures selected at random fell readily into four main groups, according to slight but constant differences in the character of the pigment as determined by a standard color scale.

3) Sports, or discontinuous variations, such as white or light colored colonies on a plate, viscosity of growth etc. sometimes occur. In general however the socalled important biological characters are not subject to discontinuous variation.

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1) Loeb, J., Further Experiments on Artificial Parthenogenesis and the Nature of the Process of Fertilization. (Amer. Jour. of Physiol. IV. 1900. p. 178.) — Matthews, A. P,, Some Ways of Causing Mitotic Division in Unfertilized Arbacia Eggs. (Amer. Jour. of Physiol. IV. 1900. p. 343.)

4) Considerable normal variation in biological characters is seen among the members of the Prodigiosus group on ordinary media. In many cases this does not exceed the variations shown by a series of inoculations made from the same culture. It was noticeable that although gas production varied for most of these organisms, the ratio of CO_2 to the total gas remained comparatively constant.

5) Within comparatively narrow limits the pigment color of the Prodigiosus group may be altered by changing the composition of the nutritive medium.

6) Also by variation in the composition of the nutritive medium, cultures usually distinct from one another in pigment character can be made to approximate, e. g. to change from orange to violet red; or to assume pigment qualities previously foreign to them, e. g. metallic luster. The former characters are regained upon transference to the original medium.

7) There is a tendency to violet red pigment in more acid, to orange red in more alkaline media.

8) Dextrose and saccharose in peptone agar medium favor pigment formation much more than does lactose.

9) The ability or non-ability to produce pigment in non-proteid media is a particularly constant character for each of the different members of the Prodigiosus group; but strains which are otherwise approximate in biological characters may differ in this ability.

10) Two strains in the Prodigiosus group, viz. B. ruber indicus I and II, differed from all the others in the ability to produce pigment in pure asparagin solution, without $MgSO_4$ or K_2HPO_4.

11) The effect upon pigment production of adding dextrose and saccharose to a standard asparagin solution is similar to that of concentrating the solution by increase of asparagin content. Here again, lactose is shown to be without effect upon pigment.

12) There is probably little or no correlation between luxuriance or vigor and the power of pigment formation. Hence pigment production does not appear to be essential to the life-processes of an organism.

D. Notes on Groups of Red Chromogenic Bacilli.

The division into groups of the pigment bacteria by means of the color scale falls in closely with the division according to other biological characters as shown by the species description table. Aside from the differentiation made in the discussion of pigment production above, the inter-relationship of the members of some groups may be briefly described as follows:

I. The Prodigiosus group.

A. Gelatin liquefied.
1. No gas in dextrose, lactose, or saccharose.
   B. prod. VIII, B. amyloruber, B. fuchsinus.
2. Gas in dextrose only.
   B. prod. I, II, III.
3. Gas in dextrose and saccharose only.
   B. prod. IV, VI, VII, B. ruber indicus I, II, B. rutilus.
4. Gas in dextrose, lactose and saccharose.
   B. prod. V, B. plymouthensis, B. miniaceus, B. kiliensis.

B. Gelatin not liquefied.
1. Gas in dextrose only.
   B. ruber miquel.

II. The Lactis erythrogenes group.

The members of this group are characterized by the production of soluble red pigment. The appended table shows also some intermediary forms described by Dyar (loc. cit.).

	Motil.	Sol. red. pig.	Insol. pig.	Gel. liq.	Agar gr.	Milk	Gr. at $37^1/_2{}^\circ$
1) B. lactis ery. I	—	agar, gel. and milk	yellow	+	lux. soft	coag. alk.	—
2) „ „ „ II	—	„ „ „ „	pale yel.	+	„ „	„ „	—
3) B. ery. rugat. Dyar	—	„ (Dyar)	yellow	+	folded	„ „	—
4) B. helvolus Zimm.	—	„ „	„	+ slowly	lux. soft	—	?
5) B. „ granul. Dyar	—	„ „	pale yel.	+ „	granular	—	?
6) B. rubefaciens	+	gel. and agar	yellowish in gel.	—	smooth	coag. acid.	—
7) B. lactorubef.	+	+ milk	white	—	„	„ „	—
8) B. rutilescens	+	gel. and agar	„	+	lux. smooth	„ „	+

A culture from Král of B. roseofluorescens Tataroff, which is said by Migula to be identical with B. lactis erythrogenes, was evidently atypical, showing thin white growth and no pigment. It was non-motile, non-liquefying, and had no effect upon milk.

III. The Rubricus group.

These cultures were of interest because they are typical forms of a group of red chromogenic organisms quite different from the Prodigiosus group. I have no doubt that the whole series of small and non-motile, non-liquefying, slow growing red forms, i. e.

distinct from B. ruber miquel, are much more closely related than the members of the Prodigiosus group, if they are not all identical. This includes forms isolated and described by Dyar, B. zeta, B. delta, B. ferrugineus, B. salmoneus, B. rhodocrous Overbeck, B. finitimus ruber, B. haematoides Wright etc. Some of these are recorded as liquefying gelatin slowly or very slowly. The pigment ranged from salmon pink and orange to red. Milk is either unchanged or alkaline.

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(See Table IX p. 46—47, Table X p. 48—51.)

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Table

Name of Organism	Source	Morphology				Cultural Features										Bio
						Nutrient broth tube		Nutrient agar tube		Gelatine plate	Gelatine stab			Potato tube		Fermentation tube
		Bacillus	Diam. greater than 1 μ	Motile	Spores	Scum	Turbidity	Dull	Wrinkled	Characteristic appearance	Deep funnel	Surface growth	Needle growth	Visible	Luxuriant	Growth in closed arm
B. prodigiosus I, II, III		+	—	+	—	±	+	—	—	+	+	+	+	+	+	+
B. prodigiosus IV, VI, VII		+	—	+	—	±	+	—	—	+	+	+	+	+	+	+
B. prodigiosus V		+	—	+	—	+	+	—	—	+	—	+	+	+	+	+
B. „ „ VIII		+	—	+	—	±	+	—	—	+	—	+	+	+	+	+
B. ruber indicus I, II		+	—	+	—	+	+	—	—	+	+	+	+	+	+	+
B. ruber plymouthensis I, II, III		+	—	+	—	±	+	—	—	+	+	+	+	+	+	+
B. kiliensis, B. ruber balticus		+	—	+	—	+	+	—	—	+	+	+	+	+	+	+
B. miniaceus I, II, III		+	—	+	—	±	+	—	—	+	+	+	+	+	+	+
B. rutilus (n. sp.)		+	—	+	—	—	+	—	—	+	+	+	+	+	+	+
B. amyloruber (n. sp.)		+	—	+	—	—	+	—	±	+	+	+	+	+	+	+
B. fuchsinus		+	—	+	—	—	+	—	—	+	+	+	+	+	+	+
B. ruber miquel		+	—	—	—	+	+	—	—	+	—	+	+	+	+	+
B. rubricus (n. sp.)		+	—	—	—	+	+	—	—	+	—	+	—	±	—	—
B. rufus (n. sp.)		+	—	—	—	+	+	—	—	+	—	+	—	+	+	—
B. ruber zimmermann		+	—	+	—	+	—	—	—	+	—	+	—	+	—	—
B. havaniensis		+	—	—	—	+	—	—	—	+	—	+	—	±	—	—
B. lactis erythrogenes I, II		+	—	—	—	+	+	—	—	+	—	+	+	+	+	+
B. rubefaciens		+	—	+	—	+	+	—	—	+	—	+	+	+	+	+
B. lactorubefaciens		+	—	+	—	+	+	—	—	+	—	+	+	+	+	+
B. rutilescens (n. sp.)		+	—	+	—	+	+	—	—	+	—	+	+	+	+	+
B. mycoides roseus		+	—	+	—	+	—	+	+	+	—	+	+	+	+	—
B. mycoides corallinus (n. sp.)		+	—	—	—	+	+	—	—	+	—	+	+	+	+	—
B. latericeus (?)		+	—	—	—	+	—	+	+	+	+	+	+	+	+	—
B. rubro pertinctus		+	—	—	—	+	—	+	+	+	—	+	+	+	+	—
B. rosaceus metalloides		+	—	+	—	+	+	—	—	+	—	+	+	+	—	—
B. mesentericus ruber, I—IV		+	—	+	+	+	+	—	—	+	—	+	+	+	+	—

IX.

logy

Biochemical Features																			Pathogenesis
Grows at body temperature	Facultative anaërobe	Affected by range of reaction	Liquefaction			Gas production			Nitrate reduced	Indol produced	Milk			Fecal odor	Nutrient agar tubes		Mice	
			Gelatin	Casein	Blood serum	Dextrose broth	Lactose broth	Saccharose broth			Curdled	Acid	Alkaline		Chromogenesis	Fluorescence	Intra-peritoneal inoculation	
+	+	+	+	—	+	+	—	—	+	—	+	+	—	—	+ lus	—		
+	+	+	+	—	+	+	—	+	+	—	+	+	—	—	+ lus	—		
+	+	+	+	—	+	+	+	+	+	—	+	+	—	—	+	—		
++	++	++	++	+	++	+	—	+	++	—	++	++	—	—	++	—		
+	+	+	+	—	+	+	+	+	+	—	+	+	—	+	+ lus	—		
+	+	+	+	—	+	+	+	+	+	—	+	+	—	—	+ lus rare	—		
++	++	++	+	—	—	+	+	+	++	—	+	+	—	—	++ lus	—		
+	++	++	+	—	+	+	—	+	++	—	+ precip.	+	—	—	+	—		
+	++	++	+	+	+	—	—	—	++	—	+	+	—	—	++ lus	—		
+	+	+	—	—	—	+	—	—	+	—	—	—	—	—	+ lus	—		
+	—	+	+	—	—	—	—	—	—	—	—	—	+	—	+	—		
++	—	++	+ s	—	—	—	—	—	—	—	—	—	++	—	++	—		
+	—	+	—	—	—	—	—	—	—	—	—	—	+	—	+	—		
+	+	+	+	+	—	—	—	—	+	—	—	+	+	+ sweet	+	+ red		
—	+	+	—	—	—	—	—	—	+	—	+	+	—	—	—	+ red		
—	+	+	—	—	—	—	—	—	+	—	—	+	—	—	—	+ red		
+	+	+	+	+	—	—	—	—	+	—	+	+	—	—	—	red		
+	—	++	—	—	—	—	—	—	++	—	—	—	+	—	+	—		
++	—	++	—	+	—	—	—	—	—	—	—	—	++	—	++	—		
++	—	++	—	—	—	—	—	—	—	—	—	—	+	—	++	—		
+	—	+	+	—	—	—	—	—	+	—	+	+	—	+	+	—		

Table

Bio

Name of Organism	Source	Morphology				Cultural Features										
						Nutrient broth tube		Nutrient agar tube		Gelatine plate	Gelatine stab			Potato tube		Fermentation tube
		Bacillus	Diam. greater than 1 μ	Motile	Spores	Scum	Turbidity	Dull	Wrinkled	Characteristic appearance	Deep funnel	Surface growth	Needle growth	Visible	Luxuriant	Growth in closed arm
B. rubidus (Eisenberg)		+	—	+	—	+	+	—	—	+	—	+	+	+	—	+
B. ruber aquatilis (Lustig)	water	+	—	+			+	—	—	+	+	+	+	+	—	
B. sardinae (Du Bois St. Sévrin)		+	—	+		+	+	—	—	+	+	+	+	+	+	
B. carneus (Tils)		+	—	+	—			—	—	+	+	+	+	+	+	
B. ruber Berolinensis (Fraenkel)	water	+	—	+	—			—	—	+		+	+	+	+	
B. sulfureus (Holzschewnikoff,		+		+	—											
B. subfulvus (Matzuschita). B. tuberigenus 4, (Gonnermann)		+		+												
B. pneumonicus agilis (Schon)		+		+	—											
B. viguali (Matzuschita) (B. Vigual)		+		+	—	+	+			+		+		+	red	
B. liquefaciens communis (Sternberg)		+		+	—			—	—	—				+		
B. pyocinnabareus (Ferchmin)	pus	+	—	—	—	+	+	—	—	+	+	+	+	wrinkl. red +	red	
B. Thévénin	pus	+														
B. ruber lactis (Conn)	milk	+	—	—	—	—	—	—	+	—	—			+	+	
B. rubiginosus (Kern)		+	+	—		+	+	—	—	+	—	+	+			
B. tuberosus (Kern)		+	—	—	—	+	+	—	—	—	+	+	+			
B. rubescens (Zimmermann)		+	—	—	—		+	—	—	+	—	+	+	dry		
B. velatus (Matzuschita)		+		—						+				+	red	
B. colere laterum	water	+	—	—	—	—	—	—	—	+	—	+	+	+		
B. exiguus (Wright)		+	—	—	—		+	—	—	—	+	+	+	+		
B. haematoides (Wright)		+	—	—	—			—	—					+		
B. epsilon (Dyar)	air	+	—	—	—			—	—							
B. zeta (Dyar)	air	+														
B. delta (Dyar)	water	+	—	—				—	—					+	—	
B. ferrugineus (Dyar)	air	+		—		—		+	—	+						
B. finitimus ruber (Dyar)		+	—	—		—		—	—	—						
B. salmoneus (Dyar)		+	—	—		—		—	—	—						

X.

logy

Biochemical Features																			Pathogenesis
			Liquefaction			Gas production					Milk				Nutrient agar tubes				Mice
Grows at body temperature	Facultative anaërobe	Affected by range of reaction	Gelatine	Casein	Blood serum	Dextrose broth	Lactose broth	Saccharose broth	Nitrate reduced	Indol produced	Curdled	Acid	Alkaline	Fecal odor	Chromogenesis	Fluorescence			Intra-peritoneal inoculation
—	+	+	+	—	—	—	—	—	—	—	—			—	brick	—			—
+	+		+		+						+ ?	+ ?	— ?		red	—			
+ red			+																
			+																
	+		+	+		+													
			+																
			+			+													
	—		+		+														
	+		+																
+ red	—		+		+						+	—	+						
+	+		+	+							+	—	+		pink				—
	+ +		+ +												yellow	red			
	+		+												+	red			
—	—		+													red			
+ —	—		+ +																
			+	+		—			+	—	+	+	—						
—			sl.			—			—		—	—							
			+ +						—		+ +	+ +	— +		pink				
			+						—		+	+	—		red				
pink			sl.								—	+							
			—						—			—	+		brick				
			—						+ sl.						reddish-pink salmon				

Table X

Name of Organism	Source	Morphology				Cultural Features											Bio
						Nutrient broth tube		Nutrient agar tube		Gelatine plate	Gelatine stab			Potato tube		Fermentation tube	
		Bacillus	Diam. greater than 1 μ	Motile	Spores	Scum	Turbidity	Dull	Wrinkled	Characteristic appearance	Deep funnel	Surface growth	Needle growth	Visible	Luxuriant	Growth in closed arm	
B. rhodochrous Overbeck (Dyar)		+	—	—		—		—	—	i							
B. rubefaciens pyogenes (Matzuschita)		+	—	+	—	+	+	—	+		—	+	+	+	+		
Bact. rubrum (Migula)		+	+	—	—			—	—	+							
Bact. erythromyxa Zopf (Migula)		+	—	—	—		+	—	—	+				+	—		
B. rubescens (Jordan)		+	—	—	—	—	—	—	—	—	—	—		—	—		
Bact. carnosum (Kern)		+	—	—	—	+	—	—	—	—	—	+	+				
Bact. roseum (Losski)		+	—	—	—	+	+	—	—	—	—	+	+	+	rose		
B. nitrogenes (Matzuschita) B. denitrificans II, Burri and Stutzer)		+		+	—	+	+	—		+	—	+	+	+	+		
B. rubescens (Matzuschita) (B. oogenes hydrosulfureus j. Zörkendörfer)		+	—	+	—	+	—	—	—		—	+	+	+	+		
B. subrubiginosus (Maschek)		+		+										+			
B. lupini (Matzuschita)		+		+										+			
B. pseudomycoides (Migula)		+		—	+			—	—	+		+	+				
B. coccineus (Pansini)		+		—	+	—	+	—	—	+	+	+	+	+	+		
B. rubellus (Okada)		+	—	+	+		+			+	—	+	+				
B. thermophilus liquef. aërobius (Oprescu)		+		—	+	+	+							+	+		
B. thermophilus liquef. tyrogenes (Oprescu)		+		—	+	+	+	—	+					+	+		
B. mycoides ruber (Matzuschita)		+		+	+									+	+		
B. Danteci (LeDantec)		+			+									+	—		
B. apicum (Canestrini)		+		+	+									+			
B. rubiginosus (Catiano)		+	—	+	+	—	—			+	—	+	+	+			
B. subcoccineus (Catiano)		+	—	+	+	—	—			+	—	+	+	+			
B. kermesinus (Tataroff)		+	+	+	+		+	+	+	—		+		+	—		
B. rosaceus margarineus (Jolles and Winkler)		+	—	+	+			—	—	+	—	+	—	+	+		
B. subrubeum (Kern)		+		—	+		+	—	—								

continued.

logy

Biochemical Features																		Pathogenesis
			Liquefaction			Gas production					Milk				Nutrient agar tubes		Mice
Grows at body temperature	Facultative anaërobe	Affected by range of reaction	Gelatine	Casein	Blood serum	Dextrose broth	Lactose broth	Saccharose broth	Nitrate reduced	Indol produced	Curdled	Acid	Alkaline	Fecal odor	Chromogenesis	Fluorescence	Intra-peritoneal inoculation
			—												pink		
+			—			—	—			+	—	+	—				+
			—												red		
+	—		—						—		—	—	—		red pink		
	+		—			+									flesh	red	
+			—		—										rose		
+			—						+						red		
—	—		—												reddish		
			—														
			++												red		
+ red			+														—
+55°	+		+		+	—	—		—	—	+	+			red		
	+		+		+	—					+						
+			+	+		—				—					red		
			+														path. for bees
			+		+												
+	+		—			—					—	—	—		red		—
+			—			—					+	+	—		orange		—
			—		—										red		
	—		—												rose		
			—														

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60) Boekhout, F. W. J., und de Vries, J. J. Ott, Ueber einen neuen chromogenen Bacillus. (Centralbl. f. Bakt. Bd. IV. 1898. p. 497.)

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61) Zimmermann, O. E. R., op. cit. as 12), Reihe I. p. 24.

B. havaniensis (Sternberg).

62) Sternberg, G. M., Manual of bacteriology. 1892. p. 718.

B. lactis erythrogenes (Hueppe).

63) Grotenfeldt, G., Studien über die Zersetzungen der Milch. (Fortschr. der Med. Bd. VII. 1889. p. 41.)
64) Tataroff [Die Dorpater Wasserbakterien. Diss. Dorpat, 1891. p. 21, p. 60?] Migula.
65) Dyar, H., On certain bacteria from the air of New York City. (Ann. N. Y. Acad. Sci. Vol. VIII. 1895. p. 324.)
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B. rubefaciens (Zimmermann).

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68) Gruber, Th., Ueber einen die Milch rosa färbenden Bacillus. (Centralbl. f. Bakt. Bd. VIII. 1902. p. 457.)

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70) Eisenberg, op. cit. as 2), p. 84.
71) Migula, op. cit., p. 482 (B. mycoides)!

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74) Grassberger, R., Ueber die nach intraperitonealer Injektion von Marktbutter bei Meerschweinchen entstehenden Veränderungen. (Münch. med. Woch. 1899. p. 343.)

B. mesentericus ruber (Globig).

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B. rosaceus metalloides (Tataroff).

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Reference list of red chromogenic Bacteria.
(cf. Table X.)

B. rubidus Eisenberg.	Bakt. Diagnostik, p. 88.	1891
B. ruber lactis (Conn).	Rep. Conn. Agric. Sta.	1899
Bact. pyocinnabareum (Ferchmin. "red pus")	Centralbl. f. Bakt. Bd. XIII. p. 103.	1894
Bact. rubiginosum (Kern)	Arb. d. techn. Hochschule zu Karlsruhe. Bd. I. Heft 4.	1896
B. rubiginosus (Catiano).	Cohns Beiträge. Bd. VII. p. 538.	1896
B. subcoccineus „	„ „ „ „ „ 539.	1896
B. coccineus (Pansini).	Virchows Archiv. Bd. CXXII. p. 437	1890
B. sardinae (Du Bois St. Sévrin).	Ann. Past. T. VIII. p. 152.	1894
B. rubellus (Okada).	Centralbl. f. Bakt. Bd. XI. p. 1.	1892
B. carneus (Tils).	Zeitschr. f. Hyg. Bd. IX. p. 294.	1890
Bact. rubrum (Migula).	Migula. p. 488.	1900
Bact. erythromyxa (Zopf).	„ p. 487.	
(Zopf himself (Ber. d. deutsch. botan. Gesellsch. 1891. p. 22), calls his form a micrococcus).		
Bact. pseudomycoides (Migula).		
Bact. carnosum (Kern) cf. Kern above also Migula. p. 485.		
Bact. tuberosum (Kern) „ „ „ „ „	p. 490.	
B. rubescens (Jordan).	Rep. Mass. State Bd. of Health. p. 835.	1890
B. roseus (Fischer)	Die Bakterien des Meeres. p. 22	1894
B. rubrofuscum (Fischer)	„ „ „ „ p. 36	
B. mesentericus roseus (Zimmermann).	Zimmermann. II. p. 26	1890
See Lustig. p. 72. Name (Kruse) see Flügge p. 303.		
Bact. roseum (Losski).	Die Mikroorganismen des Bodens. Diss. Dorpat. See Migula, p. 484.	
B. kermesinus (Tataroff).	Die Dorpater Wasserbakterien. Diss. Dorpat. See Migula p. 858.	1891
B. exiguum (Wright).	Mem. Nat. Acad. Sci. Vol. VII. p. 447.	1895
B. haematoides (Wright).	„ „ „ „ „ „ p. 448.	
B. epsilon (Dyar).	Ann. N. Y. Acad. Sci. Vol. VIII. p. 369.	1895
B. zeta „	„ „ „ „ „ „ p. 369.	
B. delta „	„ „ „ „ „ „ p. 368.	
B. ferrugineus (Dyar)	„ „ „ „ „ „ p. 361.	
B. finitimus ruber (Dyar)	„ „ „ „ „ „ p. 361.	
B. salmoneus (Dyar)		
B. rhodochrous (Overbeck. Dyar)	„ „ „ „ „ „	
(Overbeck himself called the organism a micrococcus) See his art., Nov. Act. Leop. Carol. Acad. Vol. LV. p. 16.		1891
B. ruber berolinensis (Fraenkel, p. 252)	Flügge. Die Mikroorganismen. p. 303.	1896
B. sulfureus (Holzschewnikoff).	Fortschr. d. Med. Bd. VII. p. 201.	1889
B. subfulvus (Matzuschita) = "B. tuberigenus 4",	Gonnermann, Landw. Jahrb. Bd. XXIII. p. 656.	1894
B. velatus (Matzuschita) "B. tuberigenus 5".	Gonnermann, Landw. Jahrb. Bd.XXIII. p. 657.	

B. Lupini (Matzuschita) "B. tuberigenus 7", Gonnermann, Landw. Jahrb. Bd. XXIII. p. 657.
B. pneumonicus agilis (Schou). Fortschr. d. Med. Bd. III. p. 483. 1885
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B. erubescens (Matzuschita) = B. oogenes hydrosulfureus Zörkendörfer, Arch. f. Hyg. Bd. XVI. p. 391. 1893
B. subrubiginosus Maschek.
B. rubefaciens pyogenes (Matzuschita). Centralbl. f. Bakt. Bd. XXIX. p. 378. 1901
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B. „ „ tyrogenus „ Ebenda, p. 171. 1898
B. subthermophilus (Matzuschita) = B. thermophilus IV Rabinowitsch. Zeitschr. f. Hyg. Bd. XX. p. 161. 1895
B. sacchariphilus (Laxa). Centrabl. f. Bakt. Abt. II Bd. IV. p. 362. 1898
B. mycoides ruber (B. mycoides roseus?) Matzuschita. Archiv f. Hyg. Bd. XLI. p. 251. 1901
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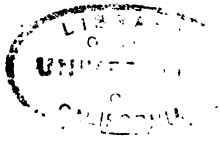
B. apicum (Canestrini) Flügge, Bd. II. p. 233.
B. mesentericus rubiginosus (Senger). Centralbl. f. Bakt. Bd. III. p. 603.



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